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From the Institute of Physiology,
University of Uppsala, Sweden

STUDIES ON THE STIMULATION MECHANISM OF GASTRIC SECRETION

BY

SVEN LINDE

UPPSALA 1950

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CHAPTER I.

Introduction.

1. The potentialities of histamine as a powerful stimulant of gastric secretion were discovered by POPIELSKI (1920). This fact has attracted the attention of many investigators. In view of the theory advanced by BABKIN (1938) that histamine may form part of the normal gastric secretory process, it is of great importance to know the exact mechanism of its secretory effect.

There is plenty of evidence to prove that histamine acts directly on the *acid*-secreting cells without the participation of the secretory nerves (Cf. BABKIN 1950). The reports about the effect of histamine on the *pepsin*-producing cells are, however, very controversial. Some authors think that histamine has a stimulating effect on the peptic cells (LIM and LIU 1926; BLOOMFIELD and POLLAND 1933; RIVERS, OSTERBERG and VANZANT 1936; GOODMAN 1938; BUCHER, IVY and GRAY 1941). Other investigators are convinced that the pepsin content of gastric juice stimulated by histamine is a pure »washing-out» product (BABKIN 1930; GILMAN and COWGILL 1931; BOWIE and VINEBERG 1935; IHRE 1938; BJÖRKMAN, NORDÉN and UVNÄS 1943).

In the present investigation attempts were made to elucidate *the mode of action of histamine on the secretory cells of the stomach*. Special interest was directed toward the activity of the peptic cells under the influence of histamine.

2. The theory of *histamine as a normal stimulant of gastric secretion* is supported by investigations in which histamine has been demonstrated in gastric juice in response to various stimuli (KOMAROW 1936; MACINTOSH 1938; CODE, HALLENBECK and GREGORY 1942; EMMELIN and KAHLSON 1944). BABKIN (1938) advanced the theory that the nervous impulses are transmitted to the parietal cells by histamine, which is liberated by acetylcholine.

Investigations into this problem would be facilitated if there existed a substance specifically antagonizing the effect of histamine on the gastric secretory cells. However, none of the antihistamine drugs so far tested have shown a significant inhibitory effect on gastric secretion induced by histamine (GROSSMAN and IVY 1946).

It has been shown recently that injection of the wetting agent Tween 20 causes urticaria and gastric secretion in dogs (GROSSMAN and ROBERTSON 1948). This secretion, which is very likely to be due to release of histamine, could be blocked by antihistamine substances.

In the present work it was assumed that Tween 20 facilitates in some way the action of antihistaminics on the gastric secretory cells. Starting from this assumption, *the effect of antihistamine substances on gastric secretion* induced by histamine or vagal stimulation was reinvestigated.

3. UVNÄS (1942) advanced the theory that *the co-operation of the pyloric part of the stomach is essential to the secretory activity of the gastric vagus nerve.* According to him, a humoral factor which is not histamine is engaged in the gastric as well as the cephalic phase of the secretory process.

This theory could not be confirmed by BABKIN et al. (1944) despite similar experimental conditions. Their results indicate that the pyloric region plays no rôle in the cephalic phase of secretion.

Because of the disagreement between these investigations the problem will be reinvestigated in the present work.

CHAPTER II.

Methods.

Animals.

All experiments in the present work were performed on dogs and cats. A description of the procedure is given here.

Dogs: Most of the experiments were performed on *dogs with* HEIDENHAIN *or* PAVLOV *stomach pouches.* The PAVLOV pouches

were prepared according to the modification of HOLLANDER and JEMERIN (1938). To avoid digestion of the surrounding skin, a Pezzer catheter was introduced through the stoma and allowed to remain there permanently.

Other experiments were performed on *anaesthetized dogs with total stomach pouches.* Combined numal-nembutal anaesthesia (0.1 ml numal and 1.0 ml nembutal per kg body weight) or in some cases chloralose-urethane anaesthesia (50 mg chloralose and 500 mg urethane per kg body weight) was used. Pylorus and cardia were ligated avoiding damage to the vagus nerve supply. A Pezzer catheter was introduced through an opening in the front wall of the pyloric region when the dog was in the prone position during the experiment. In other experiments, in which the vagi of the neck were stimulated electrically, it was more convenient to place the dog in the supine position. The catheter was then placed at the deepest point of the stomach in this position, i. e. the back wall of the fornix, and pushed out through an incision in the back.

All experiments were carried out on fasting dogs. It was always controlled for at least half an hour that there was no fasting secretion before the experiments started.

Cats: In many experiments cats with total stomach pouches were used. The anaesthetic used was a mixture of choloralose and urethane (50 mg chloralose and 500 mg urethane per kg body weight). The pylorus and cardia were ligated and a Pezzer catheter was introduced in the same way as in the anaesthetized dogs.

Technique employed in collecting gastric juice.

The gastric juice was collected in a special measuring cylinder attached to the rubber catheter (Cf. ÖBRINK 1948). This cylinder had a volume of 5 or 10 ml and was graduated in 1/10 or 1/5 of a ml. The gastric juice was allowed to drop continuously down into the cylinder, where the secretory rate could be determined at desired intervals. The cylinder was in most experiments emptied every 10 minutes.

Procedure for injection of drugs.

The drugs were administered intravenously or subcutaneously in a massive dose or by continuous intravenous injection. For the latter purpose a special injection apparatus constructed by ÖBRINK (1948) was used.

This apparatus consists of an electric motor connected with a gear box. From this box a connecting spindle leads to a syringe holder. The speed of the spindle may be changed by a factor as much as 5^6. With this device it is possible to maintain for a very long time a desired, constant concentration in the blood of the drug used. When for instance histamine is used as a stimulating agent and administered in this way, a steady state value of the secretory rate can be maintained for many hours. As will be evident later it is of the greatest importance to achieve such a steady state value in order to judge the effect of different drugs on the gastric secretion.

Technique employed in nerve stimulation.

The vagus nerves in the neck were stimulated by silver electrodes fused in a glass tube. The nerves were severed and introduced into the tube. By almost airtight closure of the tube the nerves were kept moist throughout the experiment. In order to maintain a uniformly strong stimulatory effect care was taken to move the electrodes gradually along the nerves. It was considered that the stimulation of the vagi was satisfactory when there was an inhibition of the heart rate.

The electrical stimulus was derived from the discharge of a condensor giving about 20 impulses per second. The vagi were stimulated alternately by introducing into the electrical circuit a metronome switch with a frequency of about 40 strokes per minute.

By using this stimulation technique a rather constant rate of secretion was obtained for many hours in most experiments.

Methods of analysis.

The statistical errors of the estimations are given in connection with the description below. They are expressed as the errors of single determinations and were calculated as the standard deviation from series

of 20 separate determinations on identical samples. The errors of the »standards» are also considered.

The mathematical treatment was performed according to ÖBRINK (1948).

The result (x) was calculated from a »standard» (st) with its error σ_{st} and a »sample» (sa) with its error σ_{sa} as

$$x = \frac{s\,a}{s\,t} \cdot C.$$

(C is a constant).

The error of a single determination was then calculated as

$$\sigma_x = \pm C \cdot \frac{\sqrt{s\,t^2\,\sigma_{st}^2 + s\,a^2\,\sigma_{st}^2}}{s\,t^2}.$$

1. *Total acidity of the gastric juice.*

0.1 ml of gastric juice mixed with 4 ml H_2O was titrated with 0.01 N NaOH, using *brom-thymol-blue* as an indicator (pH-range 6.0-7.6). The normality of the NaOH solution was checked against a standard of exactly controlled 0.1 N HCl in each series of determinations. As a blank 4 ml H_2O was used.

The error of the total acidity determinations was ± 2 mN.

2. *Total chlorides of the gastric juice.*

To the same samples as were used in the titration of acidity, 2 ml 0.1 N H_2SO_4 were added. The samples were then titrated electrometrically with 0.005 N $AgNO_3$, a triodometer being used. The indicator electrode was a chlorinized silver rod and the reference a calomel electrode with saturated KNO_3 in the bridge. Standard and blank were the same as in the acidity determinations. The reversal potential was determined every day from a titration curve on the standard.

The error of the total chloride determinations was ± 2 mN.

3. *Pepsin.*

In the beginning of this work several methods of estimating the activity of pepsin were considered, but none was quite

convenient. The method of ANSON and MIRSKY (1932) proved to be of high accuracy but the preparation of the substrate of carboxyhaemoglobin was very time-consuming and laborious.

However, in 1948 an ingenious method was described by HUNT (1948 a). The main principles are the same as in the method af ANSON and MIRSKY. In the method of HUNT a more readily available substrate of dried human plasma is used, which was found to be reproducible and convenient. All results published in the present work concerning pepsin determinations refer to the method of HUNT. Some modifications were performed by the author. The main principles of the method are given here.

The substrate was digested for 15 minutes with 0.5 ml of gastric juice at 35° C and pH 2.1. Unaltered protein was then precipitated by trichloroacetic acid. The amount of digestion products was determined colometrically in a COLEMAN JUNIOR spectrophotometer, using FOLIN and CIOCALTEU's (1927) phenolreagence, which reacts to tyrosine, tryptophane and cysteine groups. The blank used was a sample prepared in the same way with the exception that the gastric juice was added to the trichloroacetic acid and not to the substrate before the peptic digestion.

The peptic activity was evaluated from a curve which relates the activity to the difference in colour between the test and the blank. This curve was obtained by estimating the peptic activity in different dilutions of a sample of gastric juice with high peptic activity. In accordance with HUNT the gastric juice was said to have 50 units of peptic activity/ml when the colour was equivalent to that produced by 2.0 ml of a standard phenol solution (5.0 mg/100 ml).[1]

The error of the pepsin determinations was ± 4 units/ml.

---

[1] A commercial pepsin preparation from PARKE, DAVIS & Co. (1: 3000) was found to have a peptic activity of about 2000 units per g when determined according to the method of HUNT.

The effect of dilution of the gastric juice in the method of Hunt.

According to Bucher and Beazell (1941) there exists in the gastric juice a pepsin-inhibitor/pepsin complex dissociating on dilution. Bucher, Grossman and Ivy (1945) therefore proposed a method for pepsin determination, where the gastric juice was diluted a hundredfold in order to eliminate the effect of the inhibitor.

Hunt (1948 b) has shown, however, that no such inhibiting effect can be proved when working with his method. When the substrate was digested by undiluted gastric juice for 10 minutes or by the same juice diluted 1 in 10 for 100 minutes, the degree of digestion was the same. These results of Hunt have been confirmed by the author.

The inactivation of pepsin at lower hydrogen-ion concentrations.

It has long been known that pepsin is very unstable in an alkaline medium. Ihre (1938) found no peptic activity in gastric juice above pH 7.0 Grossman, Wooley and Ivy (1944) claimed that pepsin is inactivated irreversibly already at a pH above 5.0.

In order to elucidate the relation between the peptic activity and the pH some experiments were performed by the author. Canine gastric juice of a high pepsin content was divided into equal portions. By adding 0.1 N NaOH to each sample a desired pH was obtained. The specimens were stored for about 12 hours in a refrigerator. After that 0.1 N HCl and aq. dest. was added to bring all samples to the same pH and to the same volume. The peptic activity was then determined after storage for 3 to 4 hours at room temperature. The results are plotted together in figure 1. The highest peptic activity, obtained at pH 1.0, was taken as 100 %. Each dot in the figure represents the mean of two estimations performed on samples from two different specimens of gastric juice.

It should be emphasized that this curve represents the activity of identical amounts of pepsin but at different pH. In the experiments of the author the variations of pH were not taken into consideration. The output of pepsin was calculated from the activity of pepsin corresponding to the pH actually at hand in the analyzed samples.

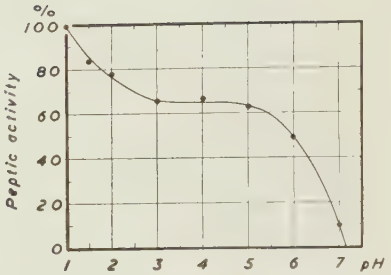

Fig. 1. The decrease of the peptic activity of canine gastric juice when stored at different pH.

The peptic activity in relation to the output of pepsin.

From results presented in chapter III of this paper it could be concluded that almost all water in the gastric juice is secreted along with HCl by the parietal cells. Pepsin is secreted with a very small amount of fluid.

Because of these circumstances there exists no parallelism between the volume secretion and the concentration of pepsin. The concentration of pepsin depends not only on the amount of pepsin secreted but also on the amount of water secreted concomitantly. The activity of the peptic cells can only be estimated by the amount of pepsin secreted per time unit.

With regard to these facts, only the output of pepsin was considered in the experiments presented in this paper.

4. *Histamine content of the plasma.*

In the very few determinations performed the method described by CODE (1937) was used.

CHAPTER III.

The mode of action of histamine on the gastric secretory cells.

A. General considerations.

Since POPIELSKI (1920) demonstrated the stimulatory effect of histamine on gastric secretion, this drug has been extensively used in experimental physiological research on the gastric secretory process. At first, several authors noted that histamine had no effect when given intravenously. GUTOWSKI (1924), however, found that when minute doses were given intravenously it was possible to stimulate the gastric secretion. TEORELL (1932, 1933) showed that continuous intravenous injection of histamine is the only method to introduce a definite concentration of the drug into the blood. When minute doses are given, one can expect a parallelism between the concentration of histamine in the blood and the rate of secretion (TEORELL 1937). ÖBRINK (1948) administered histamine by continuous intravenous injection using a specially constructed injection apparatus. He proved the existence of a simple relation between the injection rate of histamine (i. e. the histamine concentration in the blood) and the secretory rate. This relation is graphically represented by a curve passing through the origin and rising exponentially to an asymptotic value corresponding to the maximal secretion rate of the stomach pouch under histamine stimulation. With this injection technique, also used by the author, it is possible to throw more light on some problems concerning histamine stimulation of gastric secretion.

In this chapter an account is given of investigations performed by the author which demonstrate that histamine probably stimulates the parietal cells directly but has no stimulating or rather an inhibiting effect on the peptic cells.[1]

[1] According to prevailing views, the parietal cells produce hydrocloric acid and the chief cells of the body pepsin. The evidence of this is mostly of indirect nature, and direct proof is still lacking. In the present work, the view mentioned is supposed to be correct.

B. Histamine in relation to the activity of the peptic cells.

When histamine is administered to a fasting dog, there is always pepsin in the gastric juice first obtained This output of pepsin can be due to a stimulating effect of histamine on the peptic cells. But it can also be due to a »washing-out» effect, i. e. pepsin accumulated in the tubules is washed out by the parietal secretion.

A. *Many authors believe that the pepsin content of gastric juice stimulated by histamine is a pure »washing-out» product.*

Babkin (1930) believed that the peptic cells are not stimulated by histamine. After administration of a single dose of histamine to pouch dogs, the peptic power diminished rapidly when the secretion rate increased. The pepsin output also decreased to very low values. The same results were obtained whether the dog was equipped with a Pavlov or a Heidenhain pouch.

Gilman and Cowgill (1931), in experiments on Heidenhain and Pavlov pouch dogs, came to the same conclusion independently of Babkin. If a second dose of histamine was given before the effect of the first dose had worn off, it did not even temporarily increase the peptic power of the gastric juice. In some experiments they found an increased output of pepsin during the first fifteen minutes after a single dose of histamine. Like Babkin they attributed the initial relatively high peptic power to »washing-out« of the enzyme from the tubules, where it had presumably accumulated while the gastric glands were quiescent. It should be mentioned that in some experiments not only the pepsin concentration but also the pepsin output increased slightly at the end of the experiment. Gilman and Cowgill also observed a parallelism between pepsin and neutral chloride concentration in the gastric juice, and from this observation they drew the conclusion that the secretion of fixed base and that of HCl are the products of two distinct sets of glands. The eventual pH of the pure secretion as obtained from a pouch would depend upon the relative secretion rates of these two components.

Vineberg and Babkin (1931), in experiments on dogs with esophagotomy and gastric fistula, superimposed a moderate

dose of pilocarpine on the histamine stimulation. The rates of production of the fluid and the acid of the juice remained practically the same as under the effect of histamine alone, whereas after the administration of pilocarpine the chief cells began to participate actively in the secretory process. They proposed that it might be desirable to employ a combined histamine-pilocarpine test of gastric function in humans.

Bowie and Vineberg (1935) developed a method for staining the zymogen granules in the peptic cells. They showed that even after hours of secretion induced by histamine in dogs there were no signs of activity in the peptic cells. They were packed with granules. After vagal stimulation for four hours, the pepsinogen granules had disappeared almost entirely. The pepsin content of the gastric juice diminished after some hours of vagal stimulation, probably because of exhaustion of the peptic cells or damage of the vagal nerves.

In experiments on cats Björkman, Nordén and Uvnäs (1943) found that the peptic activity decreased to very low values when the animals were exposed to continuous administration of histamine. If the dose of histamine per time unit was increased during the experiment, no increase of the pepsin output was observed.

To judge from the aforementioned investigations, it seems very probable that histamine has no stimulating effect on the peptic cells.

B. *Many authors, however, are convinced that histamine has a real stimulatory effect on the peptic cells.* They do not think that the pepsin output taking place during histamine stimulation can be attributed solely to a »washing-out» effect.

Lim and Liu (1926) found pepsin in the gastric juice from Pavlov pouch dogs even after 24 hours of histamine stimulation.

Klein (1932) showed that there was pepsin even after hours of histamine stimulation in the gastric juice obtained from a subcutaneously transplanted gastric pouch deprived of its nervous and vascular supply.

Goodman (1938) applied a double histamine test on Pavlov pouch dogs and claimed that histamine had a stimulating effect on the peptic cells.

Bucher and Ivy (1941) came to the conclusion that the double histamine test is of no use for the purpose of determining whether histamine stimulates the pepsin production.

Bucher, Ivy and Gray (1941) therefore employed a »continuous histamine test» by injecting the drug subcutaneously every ten minutes on Frémont entire-stomach pouch dogs. In different series of experiments, they induced gastric secretion by supplying pilocarpine, histamine in a small dose (0.020-0.065 mg/kg/10 min.) or histamine in a large dose (0.08-0.12 mg/kg/10 min.). When the larger doses of histamine were administered, the secretory rate often decreased after a few hours. But during the second part of an eight hours' experiment, the concentration and total output of pepsin increased whatever stimulation they used. They therefore concluded, like Lim and Liu (1926), that no exhaustion of the chief cells takes place, whereas the parietal cells may be exhausted.

Another type of experiments was called the low-high-low histamine series» by these authors. After two hours of stimulation with the small histamine dose they administered the larger dose for about 3 hours, after which the lower dose was employed again for some hours. During the second period, when the large histamine dose was administered, the pepsin concentration was almost uninfluenced, but the output of pepsin rose because of the increased secretory rate. As pointed out by Babkin (1950), a possible explanation of this phenomenon is that a large dose of histamine is able to activate a greater number of the gastric glands than a small dose. It seems likely that not all the glands participate in the formation of gastric juice under the influence of submaximal stimuli (Cf. Brenckmann 1929). In the experiments of Bucher et al., the increased pepsin output during the second period can thus be explained as a »washing-out» effect. But during the third period, when a small dose of histamine was administered once more, the concentration and total output of pepsin rose markedly. The results were much the same in another series

with »low histamine-low histamine plus pilocarpine-low histamine» administration. The authors concluded that qualitatively histamine and pilocarpine have the same stimulating effect on the peptic cells.

The results of BOWIE and VINEBERG and those of UVNÄS were criticized by BUCHER (1949). She found pepsin in the gastric juice from dogs during nembutal or chloralose-urethane anaesthesia and with the vagi cut even after 9 hours' stimulation with histamine, administered every 10 minutes.

STAVRAKY (1949) came to the conclusion that histamine has a stimulating effect on the peptic cells only when there is a preexisting or combined cholinergic stimulation. He found that acetylcholine or histamine injected in the gastrosplenic artery induced a gastric secretion with low peptic power. When both drugs were given simultaneously or acetylcholine was given before the histamine, the output of pepsin was high.

The effect of histamine on the secretion of pepsin in human beings is of great importance, because the assertion that histamine stimulates the peptic cells may create the impression that histamine can be used to investigate the functional capacity of these cells. Many authors belive that the administration of histamine in the human subject produces an increased secretion of pepsin. (POLLAND 1932, BLOOMFIELD and POLLAND 1933, RIVERS, OSTERBERG and VANZANT 1936). IHRE (1938), however, came to the result that the activity of the pepsin-producing cells in the human subject depends on the vagal tonus alone; histamine has only a »washing-out» effect on pepsin.

Experimental data.

Pepsin output in longstanding experiments.

C a t s: Chloralose-urethane anaesthetized cats with total stomach pouches were used in preliminary experiments. The gastric secretion was induced by histamine administered intravenously at a continuous rate. In seven cases the histamine dose given was 1—3 mg/hour. In accordance with the results of BJÖRKMAN, NORDÉN and UVNÄS (1943) the pepsin content of the gastric juice disappeared rapidly.

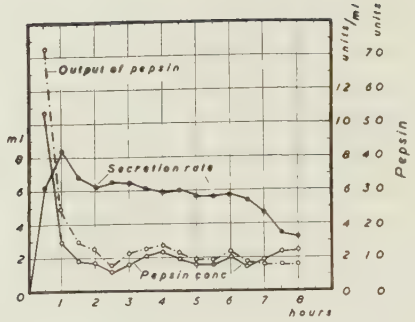

Fig. 2. Experiment showing the persistence of peptic activity in gastric juice from a cat after histamine stimulation for 8 hours.

However, in one experiment on a cat weighing 3.1 kg, where the dose of histamine was only 0.2 mg/hour, the peptic activity fell during the first hour but *there were small amounts of pepsin in the juice even after 8 hours' stimulation.* (Fig. 2).

Dogs: In 10 experiments on four HEIDENHAIN and two PAVLOV pouch dogs, varying doses of histamine were administered intravenously continuously for 3-6 hours. In most cases the peptic activity of the juice secreted was relatively high in the first samples but after that soon decreased. *In no case, however, did the peptic power fall to zero,* even if the secretory rate was maximal. It should be mentioned that the pepsin output in the HEIDENHAIN and PAVLOV pouch dogs was about the same.

In an unanaesthetized dog a continuous basal secretion of pepsin is possible even if there is no basal secretion of liquid before the histamine stimulation. As will be shown later, it is very likely that pepsin is secreted almost without fluid from the peptic cells.

The peptic activity in the gastric juice did not, however, disappear when histamine was administered even in a chloralose-urethane anaesthetized dog with total stomach pouch. To this dog, a whelp weighing 8 kg, gastric secretion was induced by giving about 0.3 mg/hour of histamine during 9 hours. The secretory rate was about 10 ml/10 minutes throughout the experiment, and the lowest value of pepsin output was 45 units/10 minutes.

From these experiments on dogs it could be concluded, either that histamine has a stimulating effect on the peptic cells in dogs or that these cells produce zymogen granules and discharge them into the lumen of the glands also in the absence of stimulation. If this last case were true, the pepsin content found in gastric juice even after very long stimulation could be explained as a »washing-out» effect.

Instillation experiments.

If there really is a continuous secretion of pepsin also in the absence of stimulation, it ought to be possible to »extract» the pepsin discharged into the crypts by instillation of HCl into the fasting stomach.

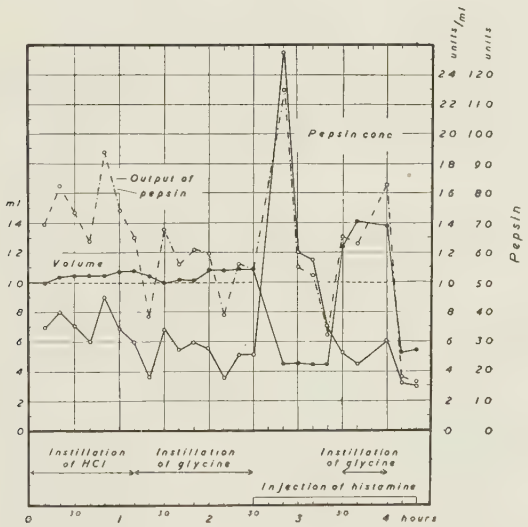

Fig. 3. The output of pepsin into HCl and glycine instilled into a Heidenhain pouch of a dog.

An experiment of this kind is demonstrated in fig. 3. During the first 70 minutes, 10 ml of HCl (170 mN) was instilled every 10 minutes into a HEIDENHAIN pouch. The dog had no secretion during a control period of one hour before the first

instillation. As seen from the figure, there was pepsin in every sample and the output of pepsin showed no tendency to decrease. The volume was not significantly changed during the instillation.

Now it seemed possible that the acid instilled might per se have some stimulating effect on the peptic cells. In the same experiment, therefore, the buffer glycine (250 mM, pH 5.6) was instilled every 10 minutes, instead of HCl, during the following 80 minutes. The peptic activity and the output of pepsin were, however, about the same as before.[1] When after that histamine was administered intravenously at a continuous rate, the pepsin output was very high in the first sample. This is the same effect as in the experiments with histamine alone described above. The most probable explanation of this effect is that the fluid instilled »extracts» pepsin only from some of the crypts but that gastric juice after histamine stimulation has a more potent »washing-out» effect.

During the last part of the experiment, glycine was instilled again simultaneously with the injection of histamine. The output of pepsin was about the same as before the injection of histamine.[2]

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[1] According to Bucher and Ivy (1941), pepsin is inactivated irreversibly at higher pH. In experiments performed by the author by adding 10 ml of glycine to 1 ml vagal stimulated gastric juice it was shown that the peptic activity was not reduced by more than 20 % when the mixture was kept in a refrigerator for 24 hours.

[2] To keep the pH on the same level during the peptic digestion by the pepsin determination in this type of experiments, 0.5 ml of HCl or 0.5 ml of glycine was added to the substrate before addition of the instilled glycine or HCl respectively.

When glycine was used, this substance was also utilized as a »dilution indicator» (Cf. Linde, Teorell and Öbrink 1947). The samples were titrated with NaOH after addition of formaldehyde. This is evidently the well known Sörensen »formaldehyde titration» and yields a value of the concentration of the aminoacid. By this method it is possible to calculate a volume increment after the instillation. In the experiment above it was shown in this manner that there was no definite volume increase in any sample of glycine after instillation when no stimulation was induced.

In four other experiments of the same type (two on the same and two on other dogs) the same results were obtained. In one of these experiments an almost maximal secretion for this dog was first obtained by giving 2 mg/hour of histamine during 70 minutes in order to »wash out» most of the pepsin accumulated in the crypts. (Fig. 4). When the instillation of

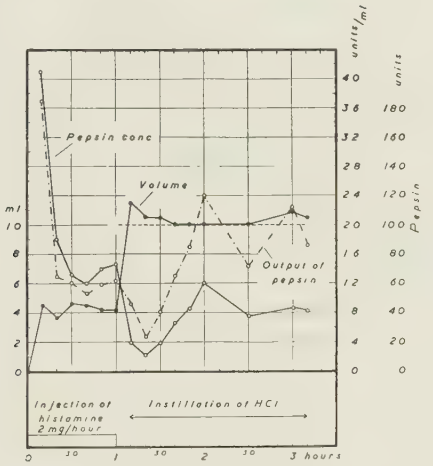

Fig. 4. The output of pepsin into HCl instilled into a Heidenhain pouch of a dog. A high dose of histamine was first injected in order to »wash out» the tubules.

HCl started, the injection of histamine was discontinued. As seen from the figure, the output of pepsin again increased when the histamine injection was stopped. During two of the last periods, the instilled HCl was left in the pouch for 30 minutes instead of the usual 10 minutes. The peptic power in these samples was about three times as large as in samples from the periods immediately before and after. In the figure the values of the pepsin output during these two periods are one third of the original values.

Summary.

From the experiments described above it may be concluded that *histamine has no stimulating effect on the peptic cells in dogs. Pepsin is probably continuously discharged into the*

lumen of the gastric glands even without stimulation. The pepsin content in gastric juice stimulated by histamine seems to be the result of a pure »washing-out» effect.

C. Inhibiting effect of histamine on pepsin secretion.

In experiments on dogs, WEBSTER (1931) found that after a subcutaneous dose of 0.5 mg histamine the decrease of pepsin concentration was not so pronounced and did not reach such a low level as after the administration of a larger dose (1 mg).

In pouch dogs and dogs with gastric fistula, ALLEY (1935) found that the total volume of secretion in response to a test meal was reduced if a preliminary secretion had been evoked by subcutaneous injection of histamine. The peptic output was also as a rule much lower if histamine had been given previously to a test meal.

One possible explanation of the results of WEBSTER and of ALLEY is that when the rate of secretion is slow some of the glands are quiescent and the »washing-out» effect weaker than if all glands participate in the secretory process. According to BABKIN (1950), however, these results may also be explained as an inhibiting action of histamine on the secretion of pepsin. As pointed out above, it seems very probable that the peptic cells continue to produce zymogen granules even in the absence of stimulation. *This continuous secretion of pepsin may perhaps be inhibited by histamine.*

Experimental data.

From several experiments published in the literature (LING, LIU and LIM 1928; GILMAN and COWGILL 1931) it is evident that after a subcutaneous injection of histamine not only the concentration but also the output of pepsin often rises at the end of the experiment in spite of the decrease in the secretory rate. This is also seen in an experiment performed by the author. (Fig. 5).

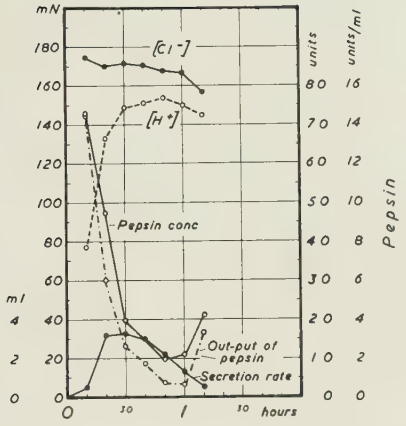

Fig. 5. The output of pepsin after injection of a single dose of histamine (0.5 mg) subcutaneously in a Heidenhain pouch dog.

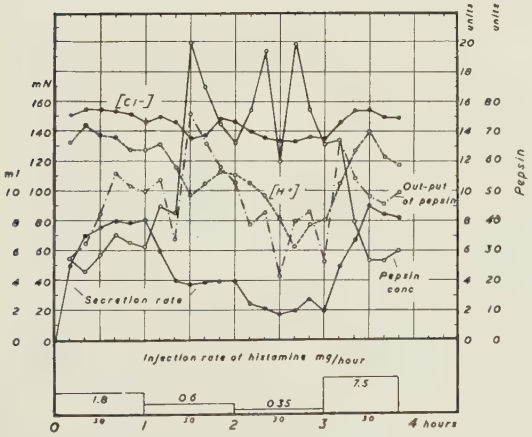

Fig. 6. The output of pepsin at different rates of histamine injection in a Heidenhain pouch dog (Dog V).

This effect is not so very easy to explain if there is no inhibiting effect of histamine on the peptic cells. To prove the correctness or incorrectness of this theory further experiments on HEIDENHAIN pouch dogs were performed.

One of these experiments is shown in fig. 6. Histamine was administered continuously by intravenous injection, but the dose injected per time unit was changed every 50-60 minutes. When the injection rate after the first hour was reduced, the peptic activity increased, and when the injection rate was higher again during the last period, the peptic activity decreased. The output of pepsin, however, showed no clear correlation to the injection rate of histamine. This is of course a result of the large variations in the secretory rate, which was reduced by about 75 % during the experiment. This experiment does not prove or disprove an inhibitory effect of histamine on the peptic cells. Even if there is such an effect, it is concealed by the »washing-out» effect, which in this experiment must be much smaller at the lower secretory rates.

Another HEIDENHAIN pouch dog, however, proved to be very convenient for this investigation. In this dog a maximal secretory rate was obtained when about 2 mg/hour of histamine was injected intravenously. When the dose of histamine was reduced to 0.8 mg/hour, the decrease of the secretory rate was, however, rather small. When very large doses of histamine were administered, the secretory rate was sometimes submaximal. This effect was also observed in other dogs and was looked upon as toxic. Four experiments of the kind described above were performed on this dog. The dose of histamine administered was varied at random from 0.8 to 14 mg/hour. These experiments are presented in fig. 7, 8, 9 and 10. All the experiments presented a high concentration and output of pepsin in the first samples, but it soon decreased. This is likely to be the result of the aforementioned »washing-out» effect. After this »washing-out» effect had worn off, there was *a close relation between the pepsin output and the dose of histamine*. When the dose of histamine was reduced to the lowest values, there was a heavy increase in the output of pepsin in spite of the reduced secretory rate. The increase of the

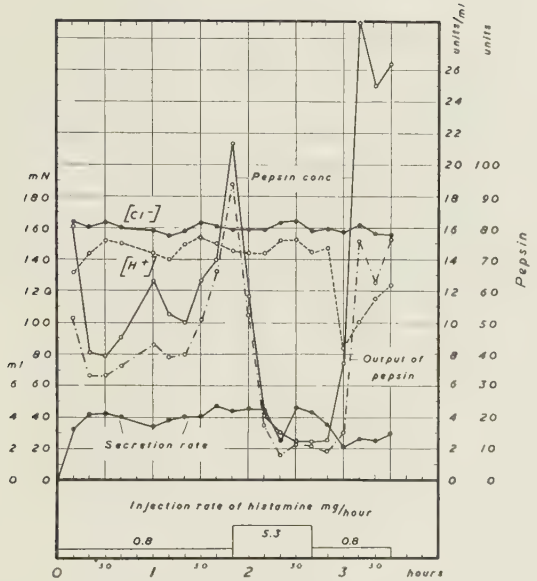

Fig. 7. The output of pepsin at different rates of histamine injection in a Heidenhain pouch dog (Dog J).

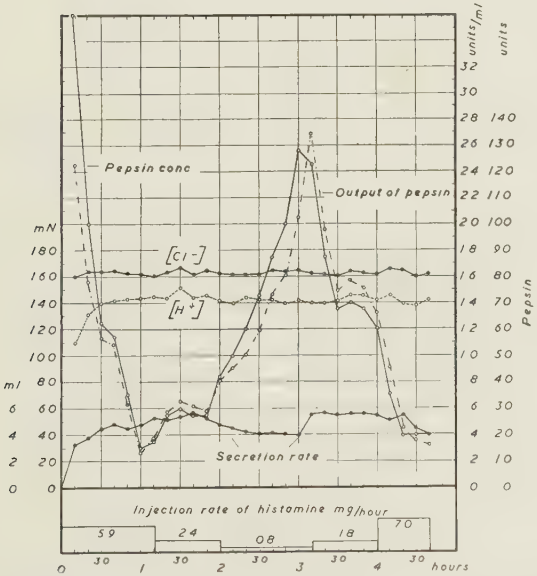

Fig. 8. The output of pepsin at different rates of histamine injection in a Heidenhain pouch dog (Dog J).

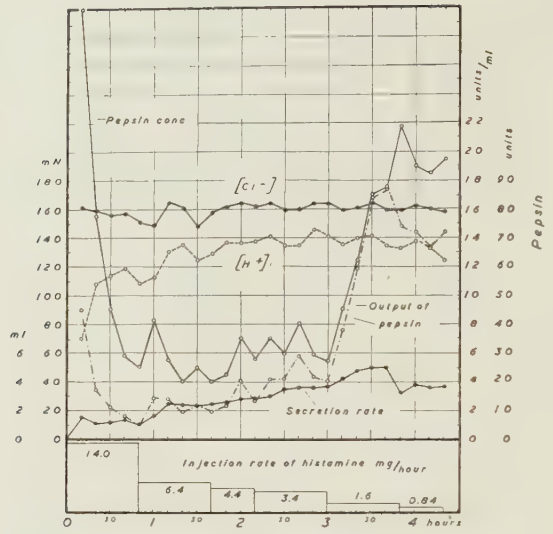

Fig. 9. The output of pepsin at different rates of histamine injection in a Heidenhain pouch dog (Dog J).

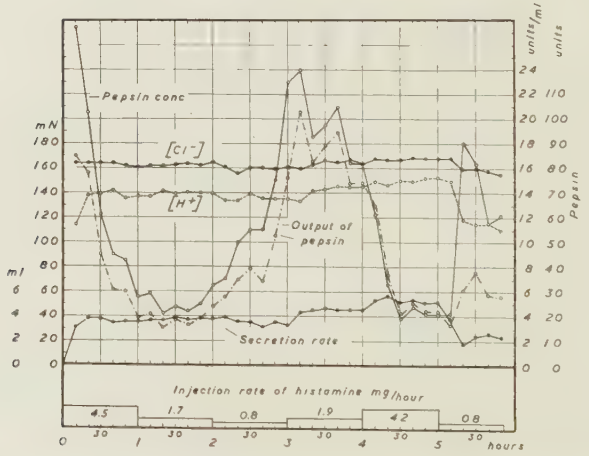

Fig. 10. The output of pepsin at different rates of histamine injection in a Heidenhain pouch dog (Dog J).

pepsin output thus cannot be the result of an increased »washing-out» effect.

As seen from the figures, the pepsin output often fell or rose steadily during the whole period of injection of the same dose of histamine. When, however, the mean of the two last values of the pepsin output during every period in all these experiments was plotted against the corresponding dose of histamine injected during the period, a close relation was obtained, as seen in fig. 11.

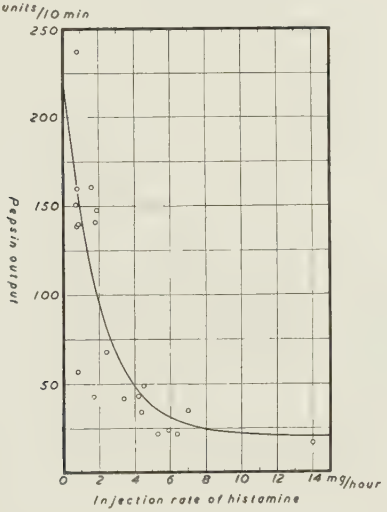

Fig. 11. The output of pepsin in relation to the injection rate of histamine. The values are obtained from four experiments in the same Heidenhain pouch dog (fig. 7-10). The smoothing curve is that obtained from eq. $y = a \cdot e^{-kx} + b$, where y denotes the output of pepsin, x the injection rate of histamine and a, k, and b are constants.

Summary.

From this figure the conclusion can be drawn that *histamine has an inhibitory effect on the peptic cells.*

D. Almost all the fluid of the gastric juice is secreted by the parietal cells.

It is very probable that the chloride concentration of the secretion of all the cellular groups of the gastric tubules, with the sole exception of the parietal cells, is equal to that of the blood, a theory advocated by HOLLANDER (1934). In other words, only the parietal cells are able to increase the chloride concentration of the gastric juice above that of the blood. As shown by many investigators, the total chloride concentration in gastric juice is the same during histamine and vagal stimulation in spite of the much higher content of organic substances and pepsin in the vagally stimulated gastric juice. If HOLLANDERS theory is accepted, the conclusion to be drawn is that almost all the water of the gastric juice is secreted by the parietal cells.

Experimental data.

In order to prove that pepsin can be secreted without accompanying fluid, the following experiments were performed.

In several chloralose-urethane anaesthetizid cats with total stomach pouch, a »supramaximal« dose of histamine was administered continuously by intravenous injection. To prove that the dose given really produced supramaximal stimulation of the parietal cells, the dose of histamine was increased by about 50 % when the secretory rate had reached a steady state level. If the secretory rate did not increase when the injection rate of histamine was increased, this was taken as a proof of a supramaximal stimulation. When given these rather large doses of histamine, many cats died, and only three of them survived the experiment. When a maximal secretory rate was obtained in these cases, the vagi in the neck were stimulated electrically in two cats. In the third cat further stimulation was induced by subcutaneous injection of pilocarpine 2.5 mg every 10 minutes. In the cases where the vagi were stimulated, the pepsin output rose tremendously to a maximal value of 289 units/10 minutes. When pilocarpine was

injected, the output of pepsin rose from zero to 23 units/10 minutes. In no case, however, did the secretory rate increase. One of the experiments with vagal stimulation is seen in fig. 12.

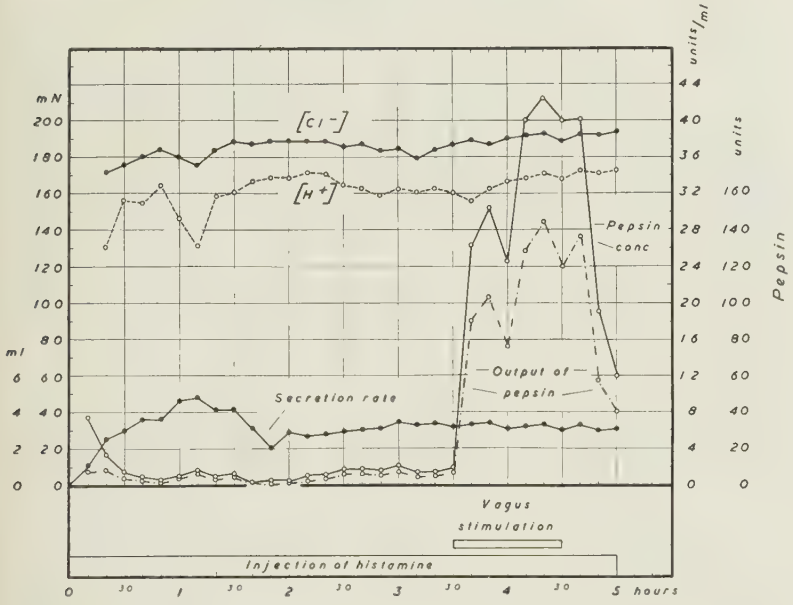

Fig. 12. The increased output of pepsin during vagal stimulation superimposed on supramaximal histamine stimulation in a cat is not followed by increased fluid secretion. (Histamine 2.5 mg/hour).

The results indicate that pilocarpine only has a small stimulating effect on the peptic cells in cats, a fact already mentioned by BAXTER (1934). In this respect the cat differs markedly from the dog. In the latter, the juice induced by pilocarpine stimulation is very rich in pepsin (BABKIN 1930, TOBY 1936).

Another experiment was performed on a HEIDENHAIN pouch dog. When a maximal secretory rate was obtained by administration of histamine, acetylcholine was injected intravenously continuously (10 mg/hour). Neither in this case was there any change of the secretory rate, but the output of pepsin rose from 20 units/10 minutes to a maximal value of 183 units/10 minutes.

Summary.

The experiments demonstrated above have shown that *pepsin can be secreted practically without accompanying fluid.*

E. The effect of atropine on gastric secretion induced by histamine.

There is plenty of evidence to prove that histamine acts directly on the parietal cells of the gastric glands. It produces secretion from a gastric pouch which is transplanted subcutaneously and deprived of the muscular layers (Klein 1932, Klein and Arnheim 1932). It even produces secretion from a totally isolated piece of mucosa (Delrue 1930, Gray et al. 1940, Teorell and Wersäll 1945, Davies 1946). Oberhelman and Dragstedt (1948), however, found that the secretory response to histamine decreased after vagotomy in dogs with total gastric pouches.

The fact that atropine does not abolish the effect of histamine speaks in favour of the theory that histamine acts directly on the acid secreting cells (Popielski 1920, Koskowski 1922, Vineberg and Babkin 1931). This fact is not, however, universally valid, for when a very small dose of histamine is given, the secretion is blocked entirely by atropine according to Lee and Kim (1937). Keeton, Koch and Luckhardt (1920) were the first to observe that atropine has some inhibitory effect on the secretion induced by histamine in dogs. It was, however, possible to induce gastric secretion by histamine even after pretreatment with almost letal doses of atropine. Polland (1930-1931), working with a »double histamine test» in human subjects, also found an inhibitory effect of atropine on gastric secretion. Gray (1937) came to the same result. He found that when histamine was administered subcutaneously in small doses every ten minutes to a Heidenhain pouch dog, gastric secretion was inhibited the more strongly the greater the dose of atropine administered. After administration of 1 mg of atropine, the secretion induced by a test meal was reduced by 100 %, while a secretion of the same magnitude evoked by histamine was reduced only by 37 %. Gray noted that in some experiments the acidity was increased after the administration of atropine. He attributed this to a more potent blocking action on the non-acid secretion than on the HCl secretion.

Oberhelman and Dragstedt (1948) found that the blocking effect of atropine on gastric secretion stimulated by histamine in dogs was the same whether the vagi were severed or not.

It should be mentioned in this connection that atropine is able to block the cephalic phase of secretion whether this is induced by vagus stimulation or sham-feeding (Uschakow 1896); the gastric and intestinal phase is also abolished (Lim, Ivy and McCarthy 1925, Ivy, Lim and McCarthy 1925). It has been reported that atropine does not abolish the action of parenterally administered secretagogues. However, Kim and Ivy (1933) showed that injection of liver extracts from which all histamine had been removed induced a secretion which could be blocked by atropine.

The fact that atropine has a much more potent inhibitory effect on the secretion after a test meal than after administration of histamine was taken in favour of the theory that gastrin and histamine are not identical substances. (Keeton et. al. 1920, Gray 1937).

As to the mode of action of atropine on gastric secretion induced by histamine there are no definite opinions in the literature. Keeton et al. (1920) concluded that the terminations of the vagi are not the sole sites upon which the drug is acting in reducing the secretion under the influence of histamine. They suggested that, in the ranges within which atropine paralyzes the secretion after food, its point of action is largely nervous; in larger doses (more than 1 mg) its action must be largely on the gland cells. Gray and Ivy (1937) suggested Dale's hypothesis as a possible solution. According to Dale (1930), atropine acts by rendering the cell impermeable to certain drugs. Employing this hypothesis, one might say that atropine prevents the access of acetylcholine to the intracellular receptive mechanism, but in the case of histamine renders the cell only partly impermeable.

As mentioned before, Stavraky (1949) thought that histamine has a stimulating effect on the peptic cells if a preexisting or simultaneous cholinergic stimulation is at hand. He also found that atropine had no blocking effect on the pepsin output if given after the administration of acetylcholine.

Experimental data.

In ten experiments on 7 HEIDENHAIN pouch dogs it was shown that atropine in sufficient doses has an inhibitory effect on the gastric secretion induced by continuous intravenous injection of histamine. When the secretory rate had reached a steady state value, atropine was injected subcutaneously in a single dose of at least 1 mg. The typical effect is seen in fig. 13. In this case the dose of histamine was about 0.75 mg/hour. After subcutaneous injection of 1.5 mg atropine, the secretory

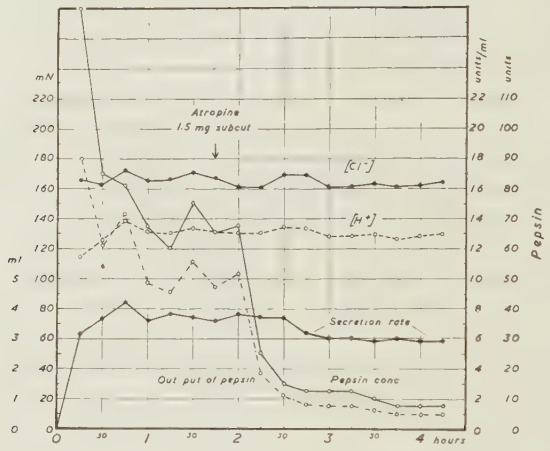

Fig. 13. The effect of subcutaneous injection of atropine on histamine stimulated gastric secretion in a Heidenhain pouch dog.

rate decreased by about 20 %. The concentration of HCl and total chlorides was unaffected. The peptic activity and output of pepsin, on the other hand, decreased rapidly to very low values.

In some cases, where larger doses of histamine were injected, the output of pepsin was relatively low even before the administration of atropine (Cf. under C above). In these cases the depressing action of atropine on the pepsin output was not so pronounced. But in all the other experiments the effect was just the same as that demonstrated in the figure above. If the secretory rate was low (in relation to the secre-

tory capacity of the pouch of the dog in question), the total acidity was also reduced after injection of atropine. This is what is to be expected from what is known about the general relations between secretion rate and acidity (TEORELL 1947: ÖBRINK 1948).

It should be mentioned that after administration of atropine in large doses, pepsin did not disappear entirely from the gastric juice except in one case, where two doses of 1.5 and 1 mg atropine were injected with an interval of 40 minutes. In one sample, pepsin was absent after the last injection. The last injection of atropine was followed by restlessness and vomiting.

The inhibitory effect of atropine on the gastric secretion stimulated by histamine is smaller the greater the dose of histamine administered. The experiments demonstrated in table 1 were performed on the same dog on different days. On each of these days, histamine was injected at a fixed rate. When a steady state value of the secretory rate was obtained, 1 mg of atropine was injected subcutaneously. The inhibitory effect on the gastric secretion was then expressed in per cent of the original value.

Table 1. **The inhibitory effect of atropine (1 mg subcutaneously) on the gastric secretion stimulated by histamine in a Pavlov pouch dog.**

Histamine; mg/hour.	Secretion before atropine; ml/10 min	Inhibition of secretory volume; %.
0.15	0.3	66
0.44	0.7	50
0.73	2.5	40
0.89	3.3	22
1.3	4.9	14
2.2	4.7	0

In this dog, the maximal secretory rate of about 5.0 ml/10 minutes was obtained by administering about 2 mg/hour of histamine. If a supramaximal histamine stimulation was applied, 1 mg of atropine had no inhibitory effect on the secretion. In one experiment on the same dog, involving administration of 3.5 mg/hour of histamine, there was no inhibition of

the secretion even after administration of 2 mg of atropine. In an other experiment, in which 6 mg/hour of histamine was administered, two doses of 2 mg and 1 mg atropine given 30 minutes after each other had no inhibiting effect on the secretion. ÖBRINK (1950) made a mathematical approach to the relation between stimulatory and inhibitory effects on gastric secretion and arrived at conclusions, that are in accordance with the results presented here.

In these experiments with supramaximal stimulation, the output of pepsin was low even before the injection of atropine, and after administration of atropine there was no reduction of the peptic activity, in contradistinction to the experiments in which smaller doses of histamine were injected. In the histamine-induced gastric juice in dogs, there thus seems to be *a rest of peptic activity which is not so easily blocked even by large doses of atropine.*

Summary.

It could be concluded that *atropine has a definite but slight inhibitory effect on the volume output after histamine stimulation in dogs.* This effect is smaller the greater the dose of histamine administered. When a dose of histamine giving a supramaximal stimulation is administered, the volume output is not inhibited.

In histamine-stimulated gastric juice in dogs, *the output of pepsin,* when high before, *is strongly inhibited by the injection of atropine.* However, there seems to be a fraction of pepsin which is very difficult to abolish even with high doses of atropine.

Discussion.

The inability of histamine to stimulate the peptic cells.

From experiments demonstrated above a strong case can be made in favour of the view that *histamine has no stimulating effect on the peptic cells in cats and dogs.*

In cats, the peptic activity in the gastric juice soon decreased to zero when not too small a dose of histamine was ad-

ministered continuously intravenously. In dogs, there was always some pepsin in the juice even in experiments of long duration. It was found, however, that with the exception of a large initial output of pepsin, there was not more pepsin in the juice under continuous histamine stimulation in dogs than in fluid which had been instilled in the pouch of the non-stimulated animal.

The effect of histamine on the peptic cells is of importance not only from a physiological point of view. If histamine has no stimulating effect in human beings either, this might create a false impression in the minds of clinicians, who might be inclined to use histamine as a means of investigating the functional capacity of these cells. The capacity of the peptic cells in human beings is of interest from several points of view. Recently much attention has been directed toward the rôle played by pepsin in the formation of ulcers (Le Veen 1947).

Spontaneous secretion of pepsin.

In experiments on dogs performed by the author, it was found that it is possible to »extract» pepsin by instilling fluid in a resting stomach pouch, even immediately after the administration of a »supramaximal» dose of histamine, which was supposed to have caused a complete »washing-out» of the pepsin accumulated in the tubules. It therefore seems justifiable to suppose that *the formation of zymogen granules in the peptic cells goes on continuously, and that some of the granules carrying pepsin are discharged in the lumina of the gastric glands during the period of secretory »inactivity»*.

The continuous secretion of pepsin is obviously not due to external factors, for in an experiment on a chloralose-urethane anaesthetized dog there was pepsin in the gastric juice even after many hours of histamine stimulation. As mentioned, Klein (1932) has demonstrated the same thing in a subcutaneously transplanted pouch.

Histological investigations have shown that the ducts of the resting gland contain a material stainable with the same dyes as the secretory granules within the cells and that they may even contain undissolved granules (Trautmann 1926).

It might be of interest to speculate on the mechanism of the spontaneous secretion of pepsin in the resting stomach. The very strong inhibitory effect of atropine on this secretion indicates that acetylcholine is involved. The ideas of FELDBERG and LIN (1949) regarding acetylcholine in the small intestine may provide an explanation. They showed that acetylcholine is released spontaneously from all layers of the gastro-intestinal canal even when the nervous structures are paralyzed. It appears from their work and those of others (HSU 1948) that acetylcholine is produced constantly in the intestine by the enzyme choline-acetylase. The acetylcholine is of course also destroyed by choline-esterase, so that the amount of free acetylcholine reflects the antagonistic activity of these two enzyme systems. The nervous structures in the wall are not responsible for this metabolism. Since there is always some free acetylcholine present, this may explain the constant activity of the peptic glands during »rest».

The inhibitory effect of histamine on the pepsin secretion.

The experimental results stated above indicate that *histamine has an inhibitory effect on the spontaneous secretion of pepsin in dogs* (Cf. fig. 11). There was not, however, a complete blocking of the secretion of pepsin even when large doses of histamine were administered.

In cats, the peptic activity soon disappeared even after a relatively small dose of histamine. It was, however, possible to demonstrate pepsin in the gastric juice also of cats after many hours of stimulation, if very small doses of histamine were used.

It is possible that the inhibitory effect of histamine is applicable to the secretion of pepsin even during e. g. vagal stimulation and sham-feeding. It seems very difficult, however, to prove this. ALLEY (1935) found that the output of pepsin in dogs was higher in the juice after sham-feeding than in juice obtained when histamine had been administered previously to the sham-feeding. In the latter case, however, the rate of secretion was much lower and therefore the »washing-out» effect not so pronounced.

Björkman, Nordén and Uvnäs (1943), on the other hand, came to the conclusion that histamine has no inhibitory effect. They first induced gastric secretion in cats by vagal stimulation only and then after a period of rest by vagal stimulation in combination with histamine injection. The amounts of pepsin in the juice of the different periods were practically the same. In estimating the results in these experiments it must, however, be remembered that during the second period with combined stimulation the volume output was much larger than during the first period. An inhibitory effect of histamine during the second period might have been masked by the more potent »washing-out» effect.

»Washing-out» effect contra inhibitory effect.

To the author it seems that the conflicting reports in the literature as to the effect of histamine on the pepsin secretion are chiefly due to the fact that *there are two factors acting in opposite directions,* which must both be taken into account. The inhibitory effect of histamine, acting on a spontaneous secretion of pepsin and resulting in a diminished output, is generally concealed by the »washing-out» effect, which like the inhibitory effect is stronger the higher the dose of histamine administered. If the all-or-none law is valid for gastric secretion induced by histamine, then part of the glands may be quiescent when a »submaximal» dose of histamine is administered. When the histamine dose is increased, the tubules of these glands are »washed-out» too.

The results demonstrated in fig. 11 originate from experiments on a dog, where one factor, viz. the »washing-out» effect, was relatively constant, because the secretory rate did not show very great variations by different doses of histamine.

In the light of these points of view it is of interest to discuss the results of some earlier workers, which are not in accordance with those of the author.

Very few investigations in this field are performed by using continuous injection of histamine. To the author it seems that no positive conclusions can be drawn from experiments in

which other modes of administering histamine are used. The »double histamine» test has been criticized by BUCHER and IVY (1941). They therefore administered histamine by subcutaneous injection every ten minutes (BUCHER, IVY and GRAY 1941) and called this method a »continuous histamine test». Strictly speaking, this name is only correct when histamine is administered in the way performed by the present author.

As mentioned in the beginning of this chapter, BUCHER et al. (1941) came to the conclusion that histamine had a stimulating effect on the peptic cells in dogs.

Firstly, they found that a large dose of histamine produced a higher output of pepsin than a small dose, but in the former case the secretory rate was about twice that found in the latter. The most likely explanation of this is that more gastric tubules were flushed out when a larger dose of histamine was used, so that more pepsin was »washed out» from them. It is of interest to note that in one dog, which was unusually refractory to histamine, so that larger doses of histamine were required, they observed the reversed response.

The second observation of importance in the work of BUCHER et al. was that the pepsin output showed a tendency to increase over the 8-hour period, at least when a high dose of histamine was administered. On the other hand, the volume output showed a tendency to decrease, an observation also made by the author in experiments of long duration (Cf. fig. 2). It is not so easy to find a probable explanation for these two phenomena. If histamine, as claimed by the author, has an inhibitory effect on the peptic cells, it is perhaps possible that these cells during histamine stimulation for such a long time became partly refractory to the inhibitory effect of histamine, while the parietal cells became refractory to the stimulating effect. If this was the case, then it would also be possible to explain the persistence of the augmented pepsin output during period 3 in the »low-high-low» histamine experiments of these authors. As mentioned in the first part of this chapter, BUCHER et al. found that the output of pepsin increased during the second period, as is to be expected considering the increased volume output. When the dose of histamine was reduced again during the third period, the high output of pepsin continued. The suggestion of the authors that this is due to an accumulative effect of histamine does not seem very likely.

By using continuous intravenous injection of histamine in cats, BJÖRKMAN, NORDÉN and UVNÄS (1943) found no stimulating effect on the pepsin production. Their results have recently been criticized by BUCHER (1949), who could not confirm them

after repeating their experiments. There are, however, in BUCHERS paper no reports about the dose of histamine given, which is of the greatest importance (Cf. experiments on cats, page 17).

Secretion of the fluid in gastric juice.

As shown above it is possible to stimulate the peptic cells to a copious secretion without additional volume secretion. The obvious inference to be drawn from these results is that *almost all the fluid of the gastric juice is secreted by the parietal cells.*

In this connection it is of interest to note that histamine, which has a very weak stimulating effect on other gland cells in the body, is able to stimulate the parietal cells to a very strong secretion of water and acid. It seems as if the stimulating effect of histamine on the parietal cells involves an other mechanism than the usual neurohumoral stimulation of different other gland cells.

It might be possible that the acid production and the secretion of water are two separate functions of the mucosa cells. The circumstances that the inhibitory action of enterogastrone is directed towards the volume output and has no specific influence on either the HCl output or the acidity regulation speak in favour of this hypothesis (ÖBRINK 1948). The close relation between volume and acidity is probably due to secondary physico-chemical processes (HALLBERG, LINDE, ULFENDAHL and ÖBRINK 1950).

If the secretion of acid is only a secondary phenomenon of the water secretion, then the chief effect of histamine in the secretory process might be that of transporting water through the mucosa.

The inhibitory effect of atropine on gastric secretion.

The very slight inhibitory effect of atropine on acid production stimulated by histamine has been taken as a support of the theory that *histamine acts directly on the parietal cells.*

There is, however, an inhibitory effect, which is not so easily explained. According to some authors (AMBACHE 1946, DANIELOPOLU 1948) histamine is able to act on living tissues by releasing acetylcholine, and the effect of this could then be inhibited by atropine. This theory is, however, severely criticized by EMMELIN and FELDBERG (1947).

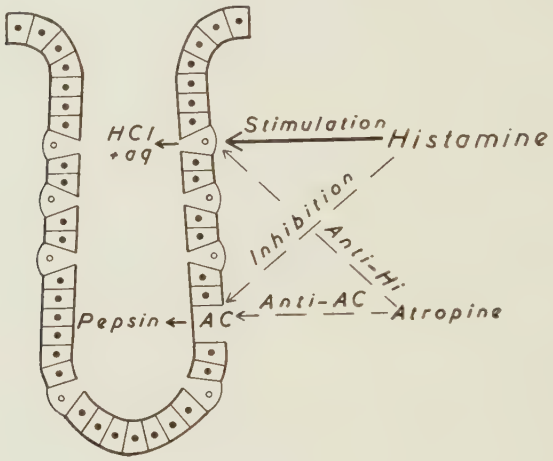

Fig. 14. The mode of action of histamine on the parietal and peptic cells of the gastric glands. AC denotes acetylcholine spontaneously released in the stomach wall.

The most probable explanation seems to be that atropine is acting on the gastric secretion induced by histamine in a twofold way. The secretion of pepsin may be blocked by an inhibition of the parasympathomimetic action of acetylcholine spontaneously released in the stomach wall. The blocking effect on the acid production is probably an antihistamine effect. It is known to be a fact that atropine is capable of antagonizing some of the effects of histamine (LOEW 1947), and the inability of atropine to inhibit the secretion during »supramaximal« histamine stimulation indicates that this assumption may be correct.

Conclusions.

Histamine has no stimulating effect on the peptic cells in dogs. It seems justifiable to suppose that a low spontaneous secretion of pepsin goes on continuously in the resting stomach.

The spontaneous secretion of pepsin is inhibited by histamine.

Histamine probably acts directly on the parietal cells. Almost all the fluid in the gastric juice is derived from these cells.

Atropine seems to have a twofold effect on gastric secretion stimulated by histamine, viz. an antiacetylcholine effect on the pepsin secretion and an antihistamine effect on the acid secretion.

All these effects are elucidated in figure 14.

CHAPTER IV.

Histamine as a normal stimulant of gastric secretion.

A. General considerations.

A considerable body of direct and indirect evidence indicates that histamine may play a rôle in the normal secretory process of the gastric mucosa.

Histamine is present in the gastric mucosa in high concentrations (Sacks, Ivy, Burgess and Vandolah 1932; Gavin, McHenry and Wilson 1933; Emmelin and Kahlson 1944). In dogs, the fundic mucosa contains more histamine than the pyloric mucosa (Gavin et al. 1933). Histaminase, though present in the intestinal mucosa, has been shown to be absent in the gastric mucosa of the dog (Best and McHenry 1930).

Histamine or a histamine-like substance has been demonstrated in gastric juice from cats and dogs in response to histamine, vagal stimulation, sham-feeding, injection of acetylcholine, eserine, mecholyl, insulin and gastrin and in juice secreted in Pavlov pouches of dogs in response to a meal (Komarow 1936; MacIntosh 1938; Code, Hallenbeck and

Gregory 1942; Emmelin and Kahlson 1944). According to MacIntosh, the histamine equivalent of the gastric juice collected during vagal stimulation is often higher than that of the blood plasma. The same author found a tendency towards a high rate of secretion to be associated with a high histamine equivalent. Such a result was not obtained in other investigations. MacIntosh could not detect any increase of the histamine equivalent of the gastric venous blood during vagal stimulation. He therefore discussed the possibility that during the cephalic phase histamine is liberated within or near to the parietal cells as a result of stimulation of the vagus. If histamine is liberated, it must be postulated that none of the histamine released enters the venous blood, because it is well known that a Heidenhain pouch will not respond to sham feeding or vagal stimulation, whereas the main stomach does secrete.

There is much evidence which strongly suggests that the chemical transmitter of the vagal secretory impulses is acetylcholine. Babkin (1938) suggested that the nervous impulse is transmitted to the parietal cells not directly by acetylcholine but by histamine which is liberated by acetylcholine under the influence of the vagus nerves.

B. The effect of Antasten on gastric secretion.

Investigations into the problem as to whether histamine plays a physiological rôle in the gastric secretory process would be facilitated if there existed a substance specifically antagonizing the effect of histamine.

Since the discovery of *histaminase* by Best (1929), several studies of the effect of this enzyme on histamine-stimulated gastric secretion in dogs have been made. In two of these investigations, the authors found an inhibitory effect on the gastric secretion induced by histamine in pouch dogs.

In the investigation of Rostorfer and Laskowski (1945), the preparation of histaminase used was, however, highly toxic. Grossman and Robertson (1948) used a preparation which was not toxic in the doses administered. They found an inhibition of the secretory response to histamine but also to

food and parasympathomimetic drugs. This might constitute strong evidence in favour of the view that histamine may be a final local excitant of gastric acid secretion produced by any type of stimulus. With regard to the work mentioned, the question arises, however, whether the secretory depressant acitivity was due to the histaminase or to some other constituent of the extract. The authors claim that »the results cannot yet be construed as evidence in favour of the hypothesis». In view of the fact that there is a marked rise in the histaminolytic power of the blood during pregnancy in human beings (AHLMARK 1944), the authors speculate on its relationship to the depression of gastric secretion (STRAUSS and CASTLE 1932) and the remission of peptic ulcer symptoms (SANDWEISS, SALTZSTEIN and FARBMAN 1939), which are known to occur during pregnancy.

In the last years a great number of substances which counteract different actions of histamine in the body have appeared. There have, however, been conflicting reports as to the action of these *antihistamine substances* on the gastric secretion induced by histamine.

The mode of action of antihistamine substances is best thought of as a competition between histamine and the antihistamine drug for a given site of action or receptive substance. It is implied that no particular action occurs if the antihistamine drug combines with the receptive substance but that it prevents thereby histamine from combining with this same site with a production of biological effect. (WELLS, MORRIS, BULL and DRAGSTEDT 1945).

LOEW and CHICKERING (1941), BURCHELL and VARCO (1942) and HALLENBECK (1943) could not demonstrate any inhibitory effect on the histamine stimulated gastric secretion in HEIDENHAIN pouch dogs by the Fourneau substances *929 F and 1571 F*. BORQUE and LOEW (1943) found a slight reduction of the gastric secretion induced by histamine and pilocarpine, but not after food stimulation. Later, LOEW (1947) seems to be doubtful about the significance of these results.

The action of *Benadryl* (β-dimethylaminoethyl benzhydryl ether hydrochloride) on gastric secretion was investigated by several authors. MCELIN and HORTON (1946) found an inhibitory effect by this drug on the histamine-induced gastric acidity in 4 out of 7 patients. MOERSCH, RIVERS and MORLOCK (1946) could not confirm these results in human

beings. LOEW, MACMILLAN and KAISER (1946) found an inhibitory effect in 3 HEIDENHAIN pouch dogs stimulated by histamine, but in the fourth dog there was no effect. SANGSTER, GROSSMAN and IVY (1946) did not find any inhibitory effect of Benadryl on gastric secretion in pouch dogs. EMMELIN and FROST (1947) came to the same conclusion in experiments on cats where the gastric secretion was induced by continuous intravenous injection of histamine.

The antihistamine substance *Neoantergan* (N-α-pyridyl-N-p-methoxybenzyl-N′, N′-dimethylethylenediamine) was found by GRAHAM (1947) to have a depressant action on the free HCl in guineapigs while the total acidity was unaffected. BOVET and WALTHERT (1944) were unable to demonstrate any inhibitory effect of this drug on gastric secretion induced by histamine in cats. WOOD (1948) came to the same result in experiments on cats stimulated by continuous injection of histamine.

SANGSTER, GROSSMAN and IVY found no inhibition of the gastric secretion from gastric pouch dogs by *Pyribenzamine* (N-α-pyridyl-N-benzyl-N′, N′-dimethylethylenediamine).

LEHMAN and NUTLEY (1949) investigated the action of the new antihistamine compound *Thephorin* (2-methyl-9-phenyl-2, 3, 4, 9-tetrahydro-1-pyridindene) upon histamine-induced gastric secretion in HEIDENHAIN pouch dogs, using a »double histamine test». They found that the secretory rate was depressed by about 30 %, whereas the acidity was unaffected.

It will be apparent from this survey that in spite of conflicting results most authors have found no inhibitory effect of antihistamine substances on histamine-induced gastric secretion. GROSSMAN and IVY (1946) stated that »none of the antihistamine drugs so far tested have shown a significant inhibitory effect on the gastric stimulating action of histamine».

On the contrary, some of the authors quoted above (LOEW and CHICKERING 1941, MCELIN and HORTON 1946, EMMELIN and FROST 1947, WOOD 1948) found an increase of the rate of secretion after administration of the antihistamine drug. None of these workers have attempted to find any explanation of this stimulating effect.

Experimental data.

In all these experiments the antihistamine drug Antasten (2-N-phenyl-N-benzylaminomethyl imidazoline) was used. According to BURN (1948) »this compound has a structural formula containing a ring similar to that of histamine and is the one

compound available, which thus has a logical basis as an antihistamine agent, for the imidazol ring probably ensures that Antasten combines with the histamine receptors and so prevents histamine molecules from exerting their effect».

The effect of Antasten on the gastric secretion stimulated by histamine was investigated in pouch dogs. 10 of these experiments were performed on HEIDENHAIN pouch dogs and 2 on a PAVLOV pouch dog. The gastric secretion was induced by continuous injection of histamine in doses giving a submaximal

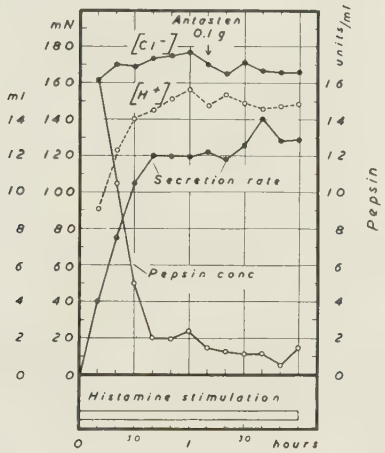

Fig. 15. The effect of subcutaneous injection of Antasten on the gastric secretion induced by histamine in a Heidenhain pouch dog.

stimulation. When the secretory rate had reached a steady value, Antasten was administered by a single subcutaneous or intravenous injection or by continuous intravenous injection. One of these experiments is demonstrated in fig. 15. As seen from the figure, there was no inhibitory effect of Antasten. On the contrary, the secretory rate increased. The output of pepsin was of about he same magnitude as before the injection of Antasten.

In none of these 12 experiments was there any inhibitory effect of Antasten. In some cases, however, there was also no stimulating effect on the volume secretion. In about half of

the experiments the pepsin output displayed a slight tendency to increase. The results are collected in table 2.

Table 2. **The effect of antasten on the gastric secretion stimulated by continuous intravenous injection of histamine in pouch dogs.**

Type of dog (Heidenhain or Pavlov pouch)	Average dose of Antasten (mg)	Mode of administration	Stimulation of volume secretion	Stimulation of pepsin secretion
H	75	S. C.	+	Not determined
H	100	S. C.	+	Not determined
H	100	S. C.	+	+
P	100	S. C.	0	+
H	100	I. V.	+	0
H	100	I. V.	+	0
H	100	I. V.	0	0
H	100	I. V.	+	0
P	100	I. V.	+	+
H	50/hour	contin.	+	+
H	100/hour	contin.	+	+
H	100/hour	contin.	0	0

The inability of Antasten (100 mg intravenously in a single dose) to inhibit the gastric secretion induced by histamine was also demonstrated in an anaesthetized dog with a total stomach pouch.

It was also shown in one experiment that the gastric secretion induced by *vagal stimulation* in an anaesthetized dog with a total stomach pouch was not inhibited by intravenous injection of 100 mg Antasten.

In a PAVLOV pouch dog gastric secretion was stimulated by continuous injection of insulin. Antasten (100 mg subcutaneously) had no inhibitory effect in this case either.

Nor was there any evidence of a stimulatory effect in any of the 3 last-mentioned experiments.

Summary.

Judging from the results of these experiments, *Antasten has per se no inhibitory effect on gastric secretion induced by histamine or vagal stimulation.* On the contrary, a stimulation of

the volume secretion induced by histamine was demonstrated in most experiments. In some experiments there seemed to be a slight stimulation of the pepsin secretion too.

C. The effect of Antasten in combination with the wetting agent Tween 20 on gastric secretion.

Ivy et al. (1948) have recently shown that the wetting agent *Tween 20,* which is a polyoxyethylene derivate of sorbitan monolaurate, produces urticaria when given intravenously in dogs.

Grossman and Robertson (1948) then found that the urticaria is accompanied by gastric secretion, which they were able to prevent or reduce by pretreatment with Benadryl. Inasmuch as Benadryl does not inhibit histamine-stimulated gastric secretion, they thought that the mode of action of Benadryl on secretion stimulated by Tween 20 must involve another mechanism. They stated that if histamine is released by Tween, Benadryl must block its formation and not its action on the gastric gland cells.

Krantz et al. (1949) found that only members of the genus Canis but no other animals tested exhibited an allergic response to Tween 20. Among the manifestations of such a response in dogs were pruritus, urticaria, erythema, increased gastric secretion, vomiting and decreased blood pressure. The depressor effect was shown to be due to the release of histamine or some histamine-like substance. The authors suggested that Tween 20 may serve as an imperfect antigen in the genus Canis.

As mentioned above, Grossman and Robertson (1948) stated that Benadryl would block the formation of histamine by Tween 20. However, one can conceive another explanation of the inhibitory effect of Benadryl on the gastric secretion induced by Tween 20. As stated above, antihistamine substances have no significant inhibitory effect on gastric secretion induced by histamine. It is probable that for some reason the antihistamine substance cannot reach the receptor elements in the gastric secreting cells. It may be possible that the »perme-

ability» of the parietal cells is increased by the action of Tween 20, after which the antihistamine drug is able to reach the cell receptors and block the histamine effect within the cells. An increased permeability produced by detergens has been found in other living tissues (BLUM and VALERIO 1946). This hypothesis as to the action of Tween 20 on the parietal cells is of course not very conclusive, but it was from this assumption that the following investigation was started.

Experimental data.

Most of the experiments were performed in the following manner.

Dogs with a total stomach pouch and anaesthetized with numal-nembutal (0.1 ml numal + 0.4 ml nembutal/kg) were used. Chloralose-urethane anaesthesia was used in some experiments on very young dogs.

Tween 20 was administered intravenously by continuous injection of an 1 % solution at a rate of 4-6 ml/hour without regard to the weight of the dog. In all 20 experiments urticaria was observed already after a few minutes. The gastric secretion started after 10-30 minutes and soon reached a maximal value. The highest secretory rate observed was 20 ml/10 minutes. In spite of continued injection of Tween, the urticaria then gradually disappeared and the secretory rate went down to zero.

At this point a continuous *intravenous injection of histamine* was started without stopping the administration of Tween. When the gastric secretion rate had reached a steady state, Antasten (in almost all cases 0.1 g) was injected intravenously in a massive dose. The time for this injection was 2-3 minutes.

In some experiments the injection of Antasten was repeated once or twice. It should be mentioned that all drugs were injected in different veins.

In an other group of experiments the gastric secretion was induced by *electrical stimulation of the vagi in the neck* instead of histamine stimulation. When the secretory rate had

obtained a fairly steady value, Antasten was injected intravenously as described above.

These are the principal traits of these experiments. More details are presented in the description of some of the experiments below.

Histamine stimulation.

Exp. 1. In fig. 16 an experiment of this kind is demonstrated. When first only Tween was injected intravenously, the stomach started secreting very soon. The secretion reached

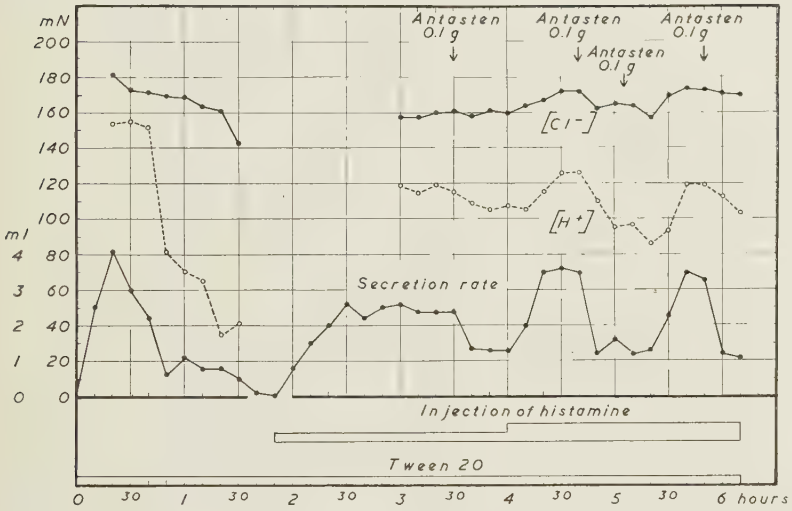

Fig. 16. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by histamine in a narcotized dog.

a maximum and then returned to zero in about 1 ½ hour. After that the continuous intravenous injection of histamine was started. When a steady state value of the secretory rate was obtained, 0.1 g of Antasten was injected slowly intravenously. There was a marked decrease of the volume output already in the first ten-minute period. The acidity also displayed a tendency to decrease. As seen from this and other experiments, the duration of the inhibitory effect was relatively short (about ½ hour).

In this experiment histamine was then injected at a higher rate, and Antasten was administered when the secretory rate was steady. The inhibitory effect was observed again. The inhibition was, however, not complete. In order to obtain a stronger inhibition Antasten was injected again when the volume output after the second injection was thought to be at its lowest value. This third injection was not, however, followed by any additional inhibitory effect. The duration of the inhibition was possibly somewhat prolonged. When the secretory

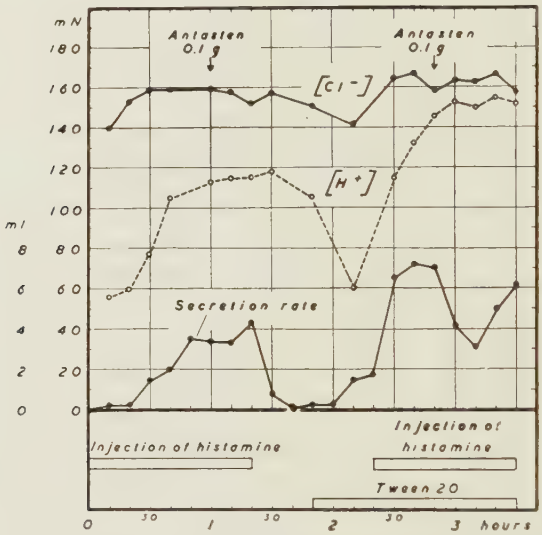

Fig. 17. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by histamine in a narcotized dog.

rate had obtained the same value as before, the inhibitory effect of Antasten was demonstrated for the third time.

In this experiment the concentration of histamine in the plasma was determined before the injection of Tween 20, during the period of secretion induced by Tween and after the disappearance of this secretion. The values obtained were 28, 62 and 32 µg/100 ml.

Exp. 2. It is seen in fig. 17 that Antasten has no inhibitory effect when first injected without Tween. When Tween was

injected in the latter part of this experiment, the inhibitory effect of the same dose of Antasten was evident.

In this case, however, it could be suspected that the stimulatory effect of Tween on the gastric secretion had not worn off when Antasten was injected. If this was the case, there could be a combined inhibitory effect on the secretion induced by Tween and by histamine. The secretion induced by Tween is inhibited by antihistamine substances, as shown by GROSSMAN and ROBERTSON (1948) as well as in some experiments performed by the present author.

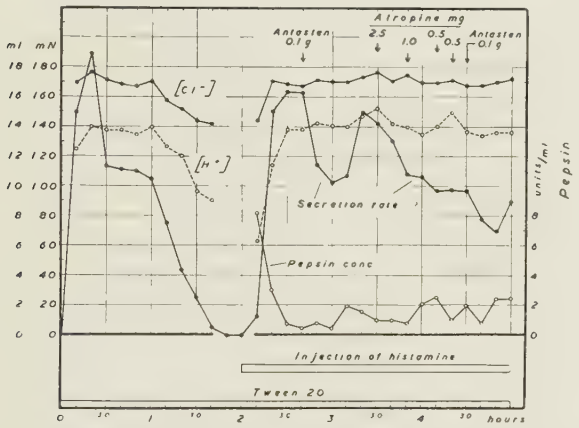

Fig. 18. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by histamine in a narcotized dog.

Exp. 3. In another experiment, demonstrated in fig. 18 the inhibitory effect was first observed after administration of 0.1 g of Antasten.

If, as suggested in chapter III, the lowering of the secretory rate by atropine is due to an antihistamine effect, it might be possible to potentiate also this effect by giving Tween. However, the inhibition caused by repeated doses of atropine administered subcutaneosly seemed to be something like that expected, and when Antasten was administered once more the inhibitory effect of this drug was about the same as after the first injection.

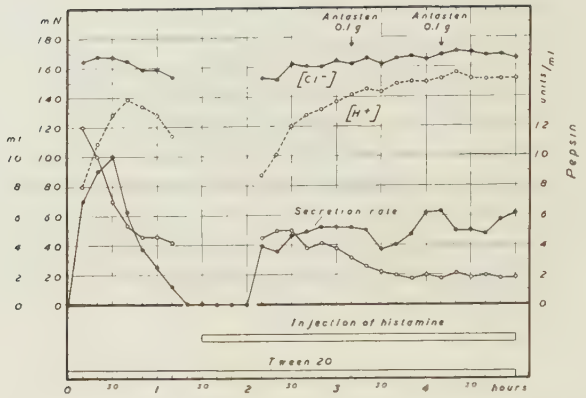

Fig. 19. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by histamine in a narcotized dog.

Exp. 4. The experiment demonstrated in fig. 19 was one of those in which the smallest inhibitory effect was observed (about 20 %). It is very difficult to find an explanation of the more pronounced effect in other experiments, where the dose of Antasten was the same and the dose of histamine and the secretory rate even higher.

Vagal stimulation.

Exp. 1. In the experiment demonstrated in fig. 20, Tween 20 was first injected as before. When the stimulating effect of Tween 20 had worn off, gastric secretion was induced by electrical stimulation of the vagi in the neck. Antasten was injected intravenously when the secretory rate was rather constant. The injection was followed by a very marked decrease of the secretory rate. There was a drop of the acidity too, but the peptic activity was practically uninfluenced. Because of the diminished volume output, the output of pepsin naturally decreased too. The same effect was observed after a second injection of Antasten.

Exp. 2. Another experiment is shown in fig. 21. In this experiment Tween 20 was not administered right from the beginning. Gastric secretion was induced by vagal stimulation. In this experiment, however, it was not so easy to obtain a

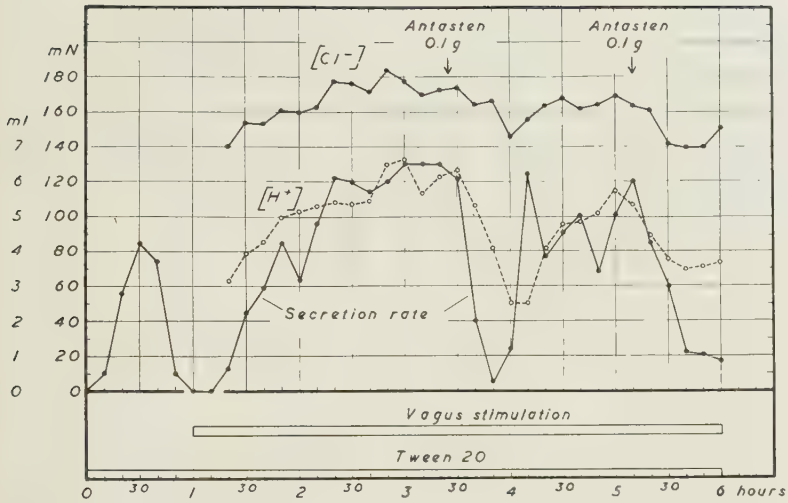

Fig. 20. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by vagal stimulation in a narcotized dog.

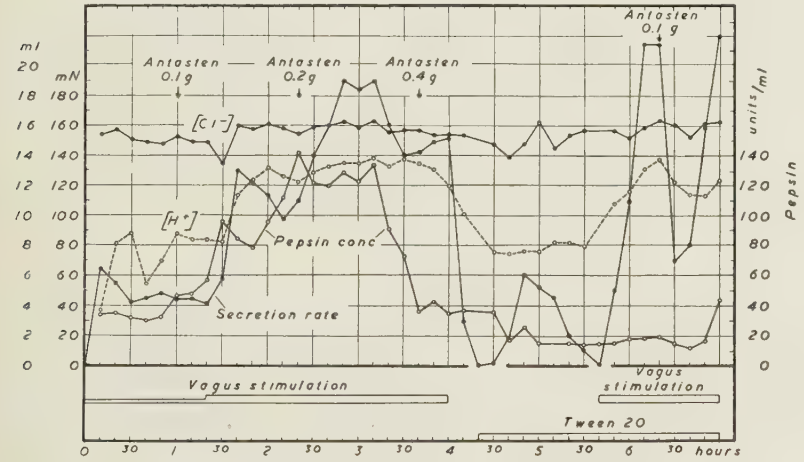

Fig. 21. The effect of Antasten per se and in combination with Tween 20 on the gastric secretion induced by vagal stimulation in a narcotized dog.

good steady state value of the secretory rate. In spite of this it was evident that there was no significant inhibitory effect on the volume output when Antasten was injected. It ought to be notized that the dose of Antasten administered the third

time was four times larger than that usually used in these experiments.

The vagal stimulation was then discontinued and Tween 20 injected continuously. When the secretion induced by Tween had returned to zero, the vagi were stimulated again. When after that Antasten was injected a pronounced inhibition of the secretory rate was observed.

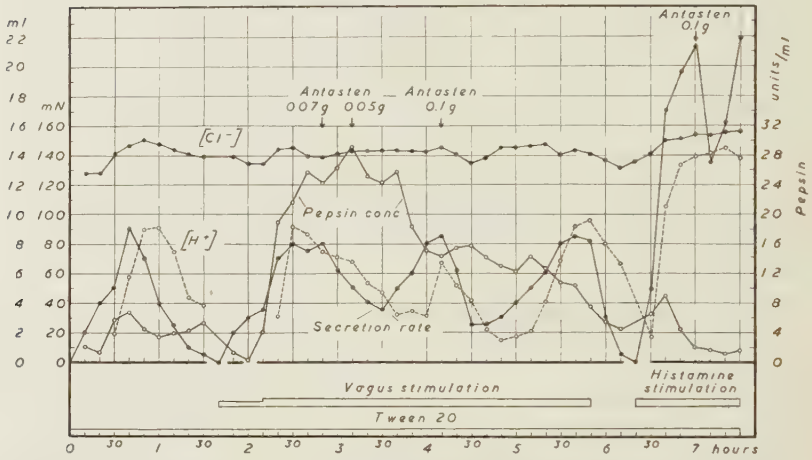

Fig. 22. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by vagal stimulation and histamine in a narcotized dog.

Exp. 3. In one experiment the inhibitory effect on gastric secretion induced by vagal stimulation and histamine injection was observed in the same dog (fig. 22). The vagi were stimulated when the secretion induced by Tween had disappeared. The inhibitory effect of Antasten on the vagal secretion was observed twice. As seen from the figure, the concentration of pepsin reached a maximum and then decreased. This is the usual behaviour in experiments involving vagal stimulation of long duration, and it is not likely to be the effect of Antasten.

After discontinuing the vagal stimulation, secretion was induced by injection of histamine. As before, it was possible to inhibit this secretion as well by injection of Antasten.

Cats: Tween 20 was injected in several experiments on cats. Neither urticaria nor gastric secretion was observed. Nor was there any inhibitory effect of Antasten on gastric secretion stimulated by histamine when Tween was injected simultaneously. The paper of KRANTZ et al. (1949) was published while these experiments were carried out, showing that only the dog and certain other members of the genus Canis exhibit an allergic response to Tween 20.

From all experiments performed by the author, the inhibitory effect of Antasten on gastric secretion induced by histamine and vagal stimulation in dogs with simultaneous administration of Tween 20 was calculated in per cent. The values are given in table 3. In this table the secretion before

Table 3. The inhibitory effect of Antasten in combination with Tween 20 on the gastric secretion induced by histamine and vagal stimulation in narcotized dogs with total stomach pouches.

Histamine stimulation			Vagal stimulation		
Secretory rate (ml/10 minutes) before inj. of Antasten	Secretory rate (ml/10 minutes) after inj. of Antasten	Inhibition (%)	Secretory rate (ml/10 minutes) before inj. of Antasten	Secretory rate (ml/10 minutes) after inj. of Antasten	Inhibition (%)
1.7	1.2	29	3.8	0 8	80
1.7	0.8	53	4.3	0.6	86
2.4	1.3	46	6.5	0.2	97
3.5	1.2	66	7.8	3.5	55
4.2	2.5	40	8.2	2.5	67
4.7	3.4	28	12.5	0.2	98
5 2	4 0	23	21.5	7.0	67
6.0	4.7	22			
6.1	3.7	39			
6.3	4.8	24			
7.1	3.1	56			
7.1	4.5	37			
7.2	6.0	17			
7.6	4.1	46			
7.9	3.5	56			
8.1	4.6	43			
10.3	8.5	17			
20.5	13.4	34			

the injection of Antasten is the mean of the two last values of the secretory rate before the injection. The secretion after administration of Antasten is the lowest value of the secretory rate in ten minutes during this period. The secretory rate after the injection of Antasten displayed large variations from one minute to another. Therefore the values of the inhibitory effect in this table are of course very approximate. For this reason, a statistical treatment of the data seems to be of very little value.

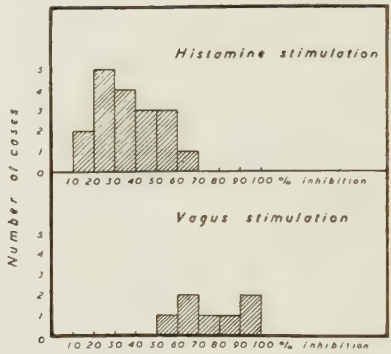

Fig. 23. The inhibitory effect of Antasten in combination with Tween 20 on gastric secretion induced by histamine in relation to that induced by vagal stimulation.

Summary.

From the figures in table 3 it is evident that there was *a significant inhibition of the gastric secretion in all the experiments.* Furthermore it may be concluded that the inhibitory effect was more pronounced on secretion induced by vagal stimulation than by histamine. (Cf. fig. 23).

Discussion.

The inability of Antasten alone to inhibit gastric secretion.

The effect of Antasten alone on gastric secretion induced by histamine and vagal stimulation was investigated and the results are demonstrated in the first part of this chapter. *No inhibitory effect was observed in any of the experiments*

performed. This is in accordance with the results of most earlier workers in this field. On the contrary, *in several experiments a stimulating effect* on the secretion induced by histamine was observed.

As to the potentiating effect of antihistamine substances on gastric secretion stimulated by histamine, there are some reports in the literature, which may provide an explanation.

It has been demonstrated that injection of antihistamine substances causes »histaminaemia». BOVET and WALTHERT (1944) thought that this might be the explanation of the aggravation of symptoms in some asthmatic patients treated with antihistamine substances. PELLERAT (1947), in a comprehensive investigation, came to the conclusion that tissue histamine is displaced by antihistamine substances from the cell receptors and is liberated in the blood. If this were so, the effect of the raise of the histamine level in the blood would mostly be hidden by the antihistamine action of the drug. In the stomach, however, the histamine liberated in the blood is able to act, while the antihistamine effect for some reason is absent.

In some experiments, however, a slight increase of the pepsin output was observed, which does not agree with a pure histamine stimulation. It has been established, however, that the enzymatic hydrolysis of acetyl-choline in the blood by choline esterase is inhibited by Antasten (PAYOT 1946). Moreover, several authors have shown that injection of eserine is followed by gastric secretion. It does not seem likely that the increased secretion after Antasten is due to an increased acetylcholine effect alone, because if this were so, an increased secretion would always be followed by a marked increase of the pepsin output.

The stimulatory effect of Tween 20.

In all experiments performed, gastric secretion was induced very soon after the injection of Tween had started. The secretion was always followed by urticaria, which indicates that the gastric secretion is due to histamine or a histamine-like substance. In all experiments in which determinations of pepsin

were performed, the peptic activity was rather low in the juice obtained during this period, as is to be expected in gastric juice stimulated by histamine.

In one experiment histamine determinations in the plasma were performed, revealing an increase of the histamine level more than twice the initial value during the period of secretion. When the secretion induced by Tween had decreased to zero in spite of continued injection of Tween, the concentration of histamine in the plasma was about the same as in the beginning.

As mentioned, the gastric secretion during administration of Tween 20 is transient. In acute allergic reactions histamine is released into the blood very rapidly, but already after 1-2 hours the histamine content of the blood is almost normal again (Code 1944). The gastric secretion during Tween administration is of about the same duration.

As mentioned, KRANTZ et al. (1949) thought that Tween acts as an antigen. The effect of Tween is not, however, necessarily due to an allergic reaction. It is possible that Tween causes a »solubilisation» and per se liberates histamine.

Inhibitory effect of Antasten and Tween 20 administered simultaneously.

Antasten administered in combination with the wetting agent Tween 20 has a marked inhibitory effect on the gastric secretion induced by histamine and vagal stimulation, as is shown in the second part of this chapter.

It is rather peculiar that antihistamine substances do not antagonize the effect of histamine on the parietal cells in the stomach mucosa, while they counteract the histamine effects in all other tissues in the body. As long as we do not know the exact mechanism of this drug antagonism, it is not easy to find an explanation of this phenomenon.

As mentioned, the hypothesis of the author when starting these experiments was that the permeability of the parietal cells may be altered by Tween 20 in such a way that the antihistamine substance is able to reach the cell receptors, which

is not the case when an antihistamine substance is given alone. This theory is of course very vague. To the author, however, it seems useless to speculate on other possible explanations. The main thing is that there is an inhibitory effect.

The specificity of the antihistamine effect.

The most important question in this discussion is *whether the inhibitory effect is really due to the antihistamine properties of Antasten.*

It is known that intravenous injection of antihistamine substances is able to cause a fall of the blood pressure of short duration (Loew 1947). It is possible that the inhibitory effect of Antasten in the experiments above is due to this hypotony. In two dogs, the blood pressure was observed throughout the experiment. There was a slight fall of the blood pressure immediatly after the injections of Antasten. However, this decrease was not more pronounced during the periods when Tween 20 was administered than in those without Tween.

In examining the results of the author, two strange features are conspicuous. One is the short duration of the inhibitory effect after Antasten, the other the more effective inhibition of the secretion induced by vagal stimulation than of that induced by histamine injection.

As to *the short duration of the inhibitory effect*, there is a possible explanation. As mentioned, histamine has been found in the gastric juice in response to different sorts of stimulation. It seems that this histamine must have passed through the parietal cells in order to excite them to activity. It is possible that such a transport through the cells takes place also in the case of Antasten. Antasten usually cannot penetrate the cells, but given in combination with Tween 20 it may possibly be transferred through them and thus be »excreted» with the parietal secretion in the same way as histamine. Such an extra elimination of Antasten from the body, caused by Tween, might explain the shortness of the inhibitory effect on gastric secretion.

The *more effective inhibitory effect of Antasten on gastric secretion induced by vagal stimulation* at first sight seems to

argue against the specificity of the Antasten effect. It is not so easy to find a probable explanation to this strange fact, but the following speculation might be of some interest. Supposing that some of the histamine in the body is bound to different elements, it can be assumed that there is normally a constant relation between the »bound» and »free histamine». If histamine is administered, there is an increase not only of the »free histamine» but also of the »bound histamine». As mentioned above, there is evidence in favour of the assumption that »bound histamine» is displaced from the receptors by Antasten and liberated in the blood. If, during continuous administration of histamine, there is more »bound histamine» available than normally, it is possible that more histamine can be liberated by Antasten. Thus the stimulating effect of Antasten on gastric secretion ought to be more pronounced under histamine stimulation than under vagal stimulation. This stimulatory effect is supposed to counteract the inhibitory effect of Antasten in combination with Tween 20. It is not so easy to prove that the stimulatory effect is greater under histamine stimulation than under vagal stimulation because of the difficulty to obtain a constant secretion in the latter case.

It is known that antihistamine substances have a nonspecific, atropine-like effect. (Halpern 1942: Meier and Bucher 1946; Loew, McMillan and Kaiser 1946). This effect may explain the fact that it is easier to inhibit gastric secretion induced by vagal stimulation than that induced by histamine. It is even possible that the inhibition of histamine-induced gastric secretion by Antasten is a specific effect but that of vagally stimulated secretion is a non-specific one. However, the ratio between the specific dose — the amount of Antasten which only blocks the effect of histamine — and the non-specific dose which inhibits the effect of spasmogenic substances is about 1: 200 (Schmiterlöv 1948). In the doses of Antasten used by the author the non-specific effect therefore seems negligible.

In some other experiments, not published above, it was found that the inhibition by atropine of the secretion stimulated by histamine was not augmented by simultaneous injec-

tion of Tween 20. This is of interest because there is evidence that atropine acts at the surface of the cells and not inside them (Cf. CLARK 1930).

Conclusions.

Antasten per se has no inhibitory effect on gastric secretion induced by histamine or vagal stimulation.

On the contrary, *Antasten seems to have some stimulatory effect on gastric secretion induced by histamine.* This effect is supposed mainly to be the consequence of a release of »bound histamine».

In combination with the wetting agent Tween 20, Antasten has a significant inhibitory effect on gastric secretion. This effect seems to be more pronounced when the secretion is induced by vagal stimulation than by histamine.

If, as supposed by the author, the inhibition is due to a specific antihistamine effect, this is strong evidence in favour of the view that *histamine is a mediator in the secretory process under vagal stimulation.*

CHAPTER V.

The co-operation between the nervous and the hormonal mechanism in the gastric secretory process.

A. General considerations.

In the preceding chapter the theory was advanced that nervous impulses are transmitted to the gastric gland cells by liberation of histamine, and that the histamine thus liberated is responsible for the secretory activity of the cells. This would mean that histamine normally acts as a gastric hormone in the cephalic phase of the secretory process.

This theory offers, however, some difficulties. The gastric juice secreted in response to histamine differs from the juice of the cephalic phase, being extremely poor in pepsin. Further-

more histamine is not the only secretagogue substance that may be extracted from the gastric mucosa. There is much evidence indicating that a gastric hormone which is not histamine operates in the gastric secretory process.

The theory of a gastric secretory hormone, »gastrin», was advanced by EDKINS (1906). It was based on the positive secretory effect produced on the gastric glands by intravenous injection of extracts of the pyloric mucosa.

As to the *gastric phase* of gastric secretion, *the existence of a humoral mechanism* was clearly indicated by the work of many early investigators (Cf. IVY 1930). The final proof of the existence of such a mechanism was the demonstration by IVY and FARRELL (1925) that the autotransplanted pouch of the fundic portion of the stomach secretes acid in response to the presence of secretagogues in the main stomach. There is furthermore much evidence in favour of the opinion that this mechanism is hormonal (GREGORY and IVY 1941; GROSSMAN, ROBERTSON and IVY 1948).

The weight of evidence now available indicates that *gastrin is not identical with histamine.* Several authors (KOMAROW 1942; UVNÄS 1943; MUNCH-PETERSEN, RÖNNOW and UVNÄS 1944; HARPER 1946; JORPES and MUTT 1950) have isolated from the pyloric mucosa a proteinlike substance, free from histamine, which is able to stimulate gastric secretion upon intravenous injection. The substance was shown to be resistant to histaminase (BAUER and UVNÄS 1944).

It seems evident that *the predominant site of gastrin formation is the pyloric mucous membrane.* It has long been recognized that stimuli for gastric secretion which act in the stomach (distension and secretagogues) are more effective when acting in the pyloric than in the fundic region (Cf. IVY 1930). It has further been demonstrated that pylorectomy reduces markedly the response of a total stomach pouch to distension and to a meal (WILHELMJ, O'BRIEN and HILL 1936; GREGORY and IVY 1941). KOMAROW (1942) and UVNÄS (1945) found that extracts from other regions than the pyloric mucosa were inactive or only produced a very slight secretion.

It is often assumed that the cephalic and gastric phases of secretion are initiated in different ways, the former by impulses conveyed by the vagi directly to the secretory cells, the latter by a combined neuro-hormonal mechanism. UVNÄS (1942), however, claimed that *also in the cephalic phase there is a co-operation between a humoral and a nervous mechanism.* It may be hypothesized that a humoral, histamine-free factor liberated from the pyloric mucosa is engaged in both the gastric and the cephalic phase of the secretory process.

The results, indicating the existence of a gastric hormone that is not histamine, do not necessarily invalidate the theory of histamine as a normal stimulant of gastric secretion. EMMELIN and KAHLSON (1944) assumed that during the cephalic and gastric phase parietal secretion is excited by a »twostage humoral mechanism», the first stage involving the liberation of gastrin, histamine being the second and final link.

B. The rôle played by the pyloric region in the secretory process during vagal stimulation.

In experiments on cats and dogs UVNÄS (1942) found that the gastric secretion during vagal stimulation is stopped or is greatly diminished by cocainization of the pyloric mucosa, by resection of the pyloric region and by tying a ligature around the pylorus so as to include the arteries supplying the pyloric region. These experimental results of UVNÄS confirmed the theory advanced by STRAATEN (1933); that the *co-operation of the pyloric part of the stomach is essential to the secretory activity of the gastric vagus nerve.*

The data, reported by UVNÄS (1942) do not agree, however, with the results obtained by some other investigators. BABKIN and SCHACHTER (1944) repeated the experiments of UVNÄS. They found that the vagal nerves are albe to convey strong secretory impulses to the gastric glands in dogs, despite total exstirpation of the pyloric part of the stomach. After pylorectomy there was usually an inhibition of gastric secretion induced by faradization of the vagal nerves, but this inhibition lasted only for some hours, after which the gastric secretion

returned to the preoperative level. The more crudely the stomach was handled, the greater and more prolonged was the inhibition of the gastric secretion. The authors presumed that the inhibition was due to hyperactivity of the sympatho-adrenal system. Intravenous injection of 50 to 100 γ of adrenaline or stimulation of the splanchnic nerves produced the same effect (BABKIN, SCHACHTER and NISSE 1944).

Since the results of BABKIN et al. (1944) do not agree with those of UVNÄS (1942) despite similar experimental conditions, it seemed of interest to reinvestigate this problem.

Experimental data.

The experiments were performed on chloralose-urethane anaesthetized cats. Special care was taken to ensure minimum damage to the tissues.

1. *Resection of the pyloric region.*

In experiments on six cats the pyloric region was resected. The resection included the distal part of the stomach from closely proximal to the angular incisure to just distal to the pylorus.

In four of these experiments, in which vagal stimulation was induced within two hours after the operation, no fluid secretion was obtained no matter how strong the stimulation was.

In two other experiments the vagi were not isolated and stimulated until about five hours after the operation. In these two cases it was possible to induce gastric secretion with the maximal secretory rates of 0.4 and 0.5 ml 10 minutes (TA 122 to 130 mN).

As observed in other experiments, a secretion of about 1.0 to 2.0 ml/10 minutes may normally be expected from this part of the stomach during vagal stimulation of the same strength.

2. *Ligation of the arteries of the pyloric region.*

The statement of BABKIN et al. (1944) that injection of adrenaline is able to inhibit the vagally stimulated gastric secretion was confirmed by the author (v i d e i n f r a). It is therefore possible that the inhibition of the secretion after the rather crude operation — pylorectomy — performed in the above experiments was due to a »hyperactivity of the sympatho-adrenal system», as suggested by BABKIN et al.

The following experiments were performed in order to attempt to prove that an inhibition of the vagally induced secretion after ligation of the arteries of the pyloric region or cocainization of the pyloric mucosa is not due to such hyperactivity.

In both series of experiments (ligation or cocainization) chloralose-urethane anaesthetized cats prepared in the following manner were used.

The pyloric region was first separated from the main stomach by a suture involving only the mucous membrane. The operation was performed by turning a portion of the stomach inside out through a small incision in the pyloric part. In this way the seromuscular junction and the nervous supply to the separated pyloric region were left intact. Through two Pezzer catheters gastric juice could be obtained from the pyloric part and the main stomach separately.

After having ascertained during at least half an hour that there was no fasting secretion, the vagi in the neck were stimulated and the secretory rate controlled for ½ to 1 hour.

In six experiments the arteries supplying the pyloric region were then ligated without stopping the vagal stimulation. The ligatures were tied as close to the stomach wall as possible and included all branches to the pyloric region from the right and left gastric arteries and from the right gastroepiploic artery.

In all cases there was an abrupt decrease of the secretory rate. The results are summarized in table 4. The secretory rates in this table are the means of two values immediately before the ligation and of two values obtained when the secretory rate was supposed to have reached its lowest point.

Table 4. **The decrease of the secretory rate after ligation of the arteries supplying the pyloric region in cats.**

Cat No.	Secretory rate before ligation ml/10 min	Secretory rate after ligation ml/10 min
1	0.4	0
2	1.3	0.5
3	1.5	0.1
4	1.5	0.1
5	1.7	0.2
6	1.8	0.1

The experiment performed on cat no. 2 is also demonstrated in figure 24. In this experiment the gastrosplenic artery was

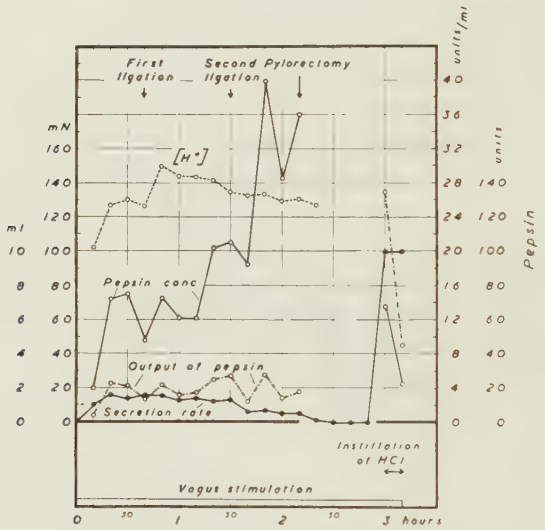

Fig. 24. Experiment showing the effect of ligation of the arteries supplying the main stomach and those supplying the pyloric region in a cat.

ligated first. It was thought that the ligation of an artery supplying chiefly the main stomach ought to cause hyperactivity of the sympatho-adrenal system if such an effect could be evoked by ligation of arteries to the pyloric region. However, no significant decrease of the secretory rate was observed after this ligation. An inhibition was not obtained until also the arteries to the pyloric region were ligated. When after

that the pyloric region was resected, the secretion decreased to zero.

It should be observed that in this case the peptic activity showed a tendency to increase when the secretory rate decreased.

3. *Cocainization of the pyloric mucosa.*

It has been shown clearly that treatment of the pyloric mucosa with cocaine or procaine causes an inhibition of the secretion induced by a meal or by distension (Savitsch 1922; Ivy 1941; Gregory and Ivy 1941). Since procaine does not inhibit the response of the parietal cells to gastrin, it has been assumed that it acts by preventing the release of gastrin (Gregory and Ivy 1941). As mentioned, Uvnäs (1942) found that secretion induced by vagal stimulation could be inhibited by cocainization of the pyloric mucosa.

The present author has performed experiments on three cats prepared in the same way as those used in the experiments in which the arteries were ligated, i. e. with the pyloric region separated from the main stomach by a suture involving only the mucosa.

After instillation of a 2 % solution of cocaine through the catheter into the pyloric part of the stomach for three minutes the secretion induced by vagal stimulation decreased in all experiments, as seen in table 5.

In the experiments on cat 1 and 2 cocaine was first instilled in the main stomach. This instillation had no significant effect on the secretory rate. After observation of the secretion for 30 to 40 minutes the second instillation in the pyloric part was performed.

Table 5. The decrease of the secretory rate after cocainization of the pyloric mucosa in cats.

Cat No.	Secretion rate before cocainization ml/10 min	Secretion rate after cocainization ml/10 min
1	1.0	0.4
2	1.1	0.3
3	1.2	0.3

4. *The effect of adrenaline on gastric secretion.*

In many experiments performed by the author it was found that adrenaline had no significant effect on gastric secretion induced by continuous intravenous injection of histamine on cats.

However, in close accordance with the results of BABKIN et al. (1944), experiments on three cats showed that the secretion induced by vagal stimulation was almost entirely blocked by intravenous injection of 50 γ adrenaline in a single dose.

Summary.

In experiments on cats it was shown that *the secretion induced by vagal stimulation was inhibited 1) by resection of the pyloric region, 2) by ligation of the arteries supplying this part of the stomach and 3) by cocainization of the pyloric mucosa.*

Ligation of the arteries supplying the main stomach or cocainization of the mucosa of this part had no significant influence on the secretion.

Intravenous injection of adrenaline caused a marked inhibition of the secretion induced by vagal stimulation.

C. The part played by the pyloric region in the secretion of pepsin.

The results demonstrated above indicate that the pyloric region presumably co-operates in the secretory process of the cephalic phase. It is, however, possible that the secretion of acid and fluid on one hand and the secretion of pepsin on the other are due to different mechanisms. The neuro-hormonal mechanism may act in the former case, whereas *the secretion of pepsin might be activated directly by nervous impulses.*

ZELIONY and SAVITSCH (1914) and SAVITSCH (1922) claimed that mechanical stimulation of the mucosa of an isolated pyloric pouch caused an augmented secretion of pepsin in the main stomach. These facts may indicate the existence of a humoral mechanism instrumental in the stimulation of peptic

secretion during the gastric phase. On the other hand Worobjew and Volborth (1934) found in Pavlov pouch dogs that the volume of secretion in response to various meals decreased after pylorectomy wheareas the peptic power increased.

Uvnäs (1945, 1948) has dicussed the possibility of the existence of a pyloric principle which stimulates the pepsin secretion. He found that crude extracts from the pyloric mucosa were able to augment the pepsin output and that therefore it might be supposed that »a principle, localized in the pyloric mucosa, would secundate the gastrin in the humoral control of the gastric secretion». He also found that the peptic cells were activated by mechanical stimulation of the pyloric mucosa.

Some investigations indicate the existence of a pepsigogue principle also in the intestinal mucosa. Babkin and Komarow (1944) found an increase of the pepsin output after administration of impure secretin preparations. Friedman et al. (1944) observed that instillation of HCl into the small intestine increased the pepsin content of the gastric juice.

The investigations of Edwards and Edwards (1948) are of interest in this connection. They found that acetylcholine in combination with eserine induced secretion of pepsin from an isolated piece of stomach mucosa.

In order to ascertain whether a principle localized in the pyloric mucosa is of importance to the secretion of pepsin during vagal stimulation, the following experiments were performed.

Experimental data.

1. *Pepsin secretion after pylorectomy.*

In the same four cats in which pylorectomy was performed and in which no fluid secretion was obtained by vagal stimulation, 10 ml HCl (170 mN) was instilled in the main stomach every ten minutes. The pepsin content of the withdrawn fluid was then determined.

One of these experiments is seen in fig. 24. Acid was instilled after pylorectomy. The concentration of pepsin in the instilled acid was relatively high and the output of pepsin even higher than in the juice obtained before the resection of the pylorus.

In two other experiments, one of which is demonstrated in figure 25, acid was first instilled into the main stomach after pylorectomy without stimulation of the vagi. The vagi were

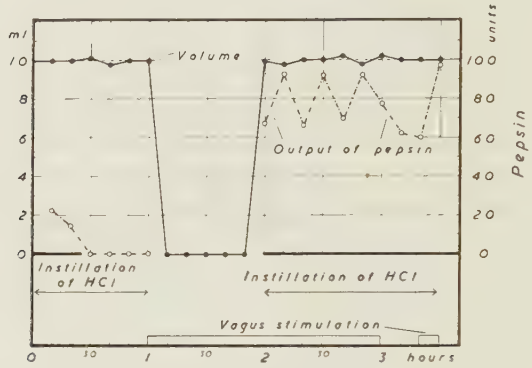

Fig. 25. The output of pepsin into HCl instilled in the stomach of a cat after pylorectomy.

then stimulated during a period without instillation of HCl. No fluid secretion was observed. Acid was then instilled again without stopping of the vagal stimulation. In both cases the output of pepsin during the last period was very high (the maximum values were 48 and 98 units/10 minutes) in contradistinction to the very low output during the first period.

In the fourth experiment acid was instilled already before the resection of the pyloric part. The vagi were stimulated during this period and throughout the experiment. The output of pepsin in the acid instilled after the pylorectomy was somewhat lower in this case (72-63-81 units/10 minutes) than in the acid instilled from the beginning (95-82-108 units/10 minutes).

In an additional experiment the pyloric region was resected and acid instilled as before. Acetylcholine was then injected continuously into the gastrosplenic artery (20 mg/hour). Despite the absence of fluid secretion the output of pepsin into the instilled acid rose from 0 to abour 75 units/10 minutes.

2. *Pepsin secretion after ligation of the arteries supplying the pyloric region.*

In four of the experiments in which the arteries supplying the pyloric region were ligated HCl was instilled into the main stomach as described above.

In these cases the secretory rate had decreased to 0 or 0.1 ml/10 minutes. When acid was then instilled, the output of pepsin was higher than in the juice obtained before the ligation in three experiments. In the fourth case the output was about the same during the two periods. One of these experiments is seen in figure 26.

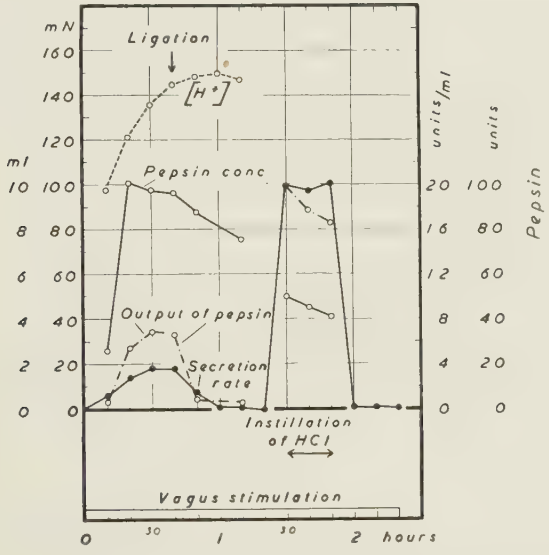

Fig. 26. The output of pepsin before and after ligation of the arteries supplying the pyloric region of a cats stomach.

Summary.

The results shown above indicate that *the pyloric region is not essential to the secretion of pepsin during vagal stimulation.* In experiments in which no fluid secretion was induced by vagal stimulation after pylorectomy or ligation of the arteries of the pyloric region, a large output of pepsin was obtained in an acid solution instilled into the main stomach.

D. The secretagogue effect of gastrin.

It has been suggested that the secretory principle of the pyloric mucosa, though not histamine, stimulates the parietal cells but not the peptic cells of the gastric glands. (KOMAROW 1942; UVNÄS 1945). It has also been shown that atropine does not abolish the effect of gastrin.

To confirm that gastrin has an effect similar to that of histamine the following experiments were performed.

Experimental data.

Gastrin, prepared by E. JORPES and V. MUTT (Chemistry Departement II, Caroline Institute, Stockholm, Sweden) was used.

The preparation was shown to be highly active. According to JORPES and MUTT, continuous intravenous injection of 10 mg during 10 minutes in a vagotomized, chloralose-urethane anaesthetized cat with a total stomach pouch was followed by a secretion of 10-20 ml 0.1 N HCl. By testing the effect on cat's blood pressure the extract was shown to have no histamine activity.

Two experiments were performed on a PAVLOV pouch dog and two on a HEIDENHAIN pouch dog. Gastrin was injected intravenously continuously at a rate of 25 mg/hour. The maximal secretory rate varied between 0.7 and 1.7 ml/10 minutes in the various experiments. In two of these experiments the secretion remained unchanged during the observation time of 30-40 minutes. In the other two cases there was a slight decrease of the volume output after an initial maximum value in the first ten-minute periods. *The output of pepsin was low in all experiments.*

In one dog 1.0 mg of atropine was administered subcutaneously during the injection of gastrin. This experiment is demonstrated in fig. 27. This dog, a PAVLOV pouch dog, was the same as that used in the quantitative estimation of the inhibitory effect of atropine on gastric secretion stimulated by histamine, as demonstrated in table 1, page 33. As seen when comparing this table with figure 27, *the inhibition of the secretory volume by atropine was of about the same magnitude, whether the secretion was induced by histamine or by gastrin.*

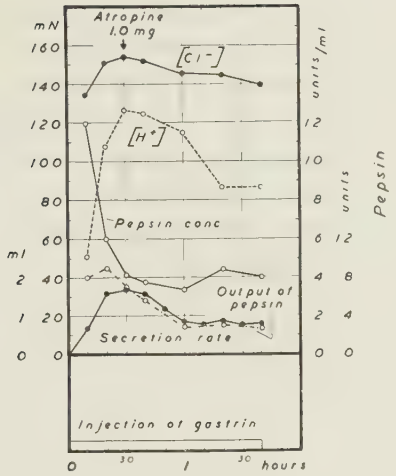

Fig. 27. Gastric secretion during continuous injection of gastrin in a Pavlov pouch dog.

Summary.

Gastrin injected intravenously at a continuous rate in dogs initiated a secretion poor in pepsin. The inhibitory effect of atropine was of about the same magnitude as on secretion induced by histamine. Thus *gastrin seems to have an effect similar to that of histamine* on the gastric glands.

E. The synergistic action of acetylcholine and histamine or gastrin on the gastric glands.

The two mechanisms — nervous and pyloric hormonal — which regulate the activity of the gastric glands had always been thought to be able to work independently of each other.

Uvnäs (1942), however, claimed that, when nervous and humoral impulses act in conjunction, they produce a strong action of the gastric glands, but that, when acting separately they produce almost no secretion. He found that stimulation of the vagi or intravenous injection of pyloric extracts in animals

whose pyloric region was resected produced a scanty secretion; when applied concomitantly, they produced an abundant secretion.

The theory of UVNÄS implies that stimulation of the vagus nerves affects both the gastric glands, which secrete gastric juice, and the pyloric mucosa, which secretes gastrin. Gastrin is conveyed by the blood to the glands of the fundus and the corpus, there greatly enhancing the effect of vagal stimulation of these glands.

This theory has met critizism (BABKIN 1950; GROSSMAN 1950). The main object of this critizism is the statement that each mechanism is a s i n e q u a n o n for the secretory process. There is in fact much evidence in favour of the assumption that *the two mechanisms are able to work independently of each other.*

Firstly, it is possible to produce a considerable secretion of gastric juice in response to intravenous injection of small doses of gastrin. Further it has been demonstrated that mechanical distension of the stomach stimulates secretion in a transplanted pouch (IVY and FARRELL 1925; LIM, LOO and LIU 1927). This shows that in the chemical phase the gastrin mechanism is able to operate in complete absence of the vagus nerve.

On the other hand the integrity of the pyloric part is evidently not a s i n e q u a n o n for the production of the secretory effect of the gastric vagus nerve. This is made evident by, among other things, the experiments demonstrated above by the present author.

The statement that the nervous and pyloric hormonal mechanism are able to work independently of each other does not, however, invalidate the theory of UVNÄS that there exists a co-operation between them. The view that vagal stimuli and gastrin can act together in stimulating gastric secretion is certainly in accord with the observations made, in the sence that either of these two mechanisms can act together *additively.* The theory of UVNÄS, however, involves an effect far beyond a simple addition, i. e. a *synergistic* action of the two mechanisms. According to UVNÄS, neither the vagal nor the gastrin mechanism can operate to its full extent in the absence of the

other during the nervous phase of secretion. This view is supported by some other investigations in the literature, which indicate that *the combined action of cholinergic and hormonal stimuli is not merely additive but may be synergistic.*

Like those of the other parasympathetic nerves, the impulses from the vagus are most probably conveyed to the stomach by a »chemical transmitter», viz. acetylcholine. This was proved in the case of the muscles of the stomach by DALE and FELDBERG (1934). No proof exists as yet with regard to the secretory function of the stomach. However, the fact that the gastric glands produce a »vagal» type of secretion in response to acetylcholine (NECHELES et al. 1938) strongly suggests that the chemical transmitter of the vagal secretory impulses is acetylcholine.

The effect of acetylcholine on the gastric secretion has been studied by numerous investigators with varying results. The investigations of NECHELES, MOTEL, KOSSE and NEUWELT (1938) finally established that very large subcutaneous doses of acetylcholine (e. g. 400 mg) had to be used in order to produce gastric secretion from a HEIDENHAIN pouch in a dog. Secretion was also induced by repeated injections of small doses (10 to 100 mg) every ten minutes. Such large doses of acetylcholine were required because of its destruction by esterase. When a small dose of acetylcholine was administered together with prostigmine, a copious secretion was obtained.

VINEBERG (1936) injected acetylcholine directly into the gastric arteries of dogs and obtained a thick mucous fluid, which was rather rich in pepsin, as in the experiments of NECHELES et al. (1938).

STAVRAKY (1945) found that the juice obtained by injection of acetylcholine into the splenic artery was devoid of free acid and pepsin. The juice obtained by injection of histamine in the same way was highly acid but poor in pepsin. However, both drugs given concomitantly produced a typical vagal juice rich in pepsin.

According to NECHELES et al. (1938), histamine and acetylcholine have a synergic effect on the gastric glands. A slow secretion evoked by one of these drugs was greatly increased by the concomitant administration of the other.

The above investigations indicate that acetylcholine and histamine might have a synergistic effect on the gastric glands.

To prove that the combined effect is more than additive, i. e. really synergistic, the author performed some experiments using the continuous injection technique.

Experimental data.

1. *The stimulating effect of acetylcholine.*

These experiments were performed on two HEIDENHAIN and one PAVLOV pouch dog.

In 6 experiments acetylcholine was injected intravenously at a continuous rate. When 25 to 50 mg/hour of acetylcholine was administered, the maximal secretory rate reached 0.3 ml/10 minutes. When in one case 60 mg/hour was injected, the maximal secretory rate was 0.5 ml/10 minutes. The secreted juice was thick and mucous with low acidity. The peptic power, determined in a mixture of all samples from each experiment during the acetylcholine stimulation, was high (13-47 units/ml) in all cases but one, thus in accordance with the results of VINEBERG (1936) and NECHELES et al. (1938) but in opposition to those of STAVRAKY (1945).

2. *The combined action of acetylcholine and histamine.*

In 11 experiments a steady state level of the secretion was first obtained by continuous injection of histamine in a dose producing a submaximal stimulation. Acetylcholine was then administered continuously intravenously concomitantly with the injection of histamine. *In all experiments but one there was an increase of the secretory rate which was higher than that expected if the combined stimulatory effect had been merely additive.*

Two of these experiments are demonstrated in figure 28 and 29. As seen, in one of these experiments the secretory rate during the injection of acetylcholine reached a maximum and then decreased again. This was observed in several experiments. The same observation was made by GRAY and IVY (1937) during combined administration of mecholyl and histamine.

In all experiments there was a great increase of the pepsin output when acetylcholine was administered.

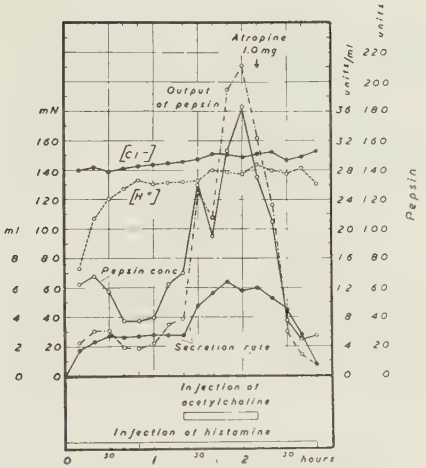

Fig. 28. The potentiating effect of acetylcholine superimposed on histamine stimulation in a Heidenhain pouch dog.

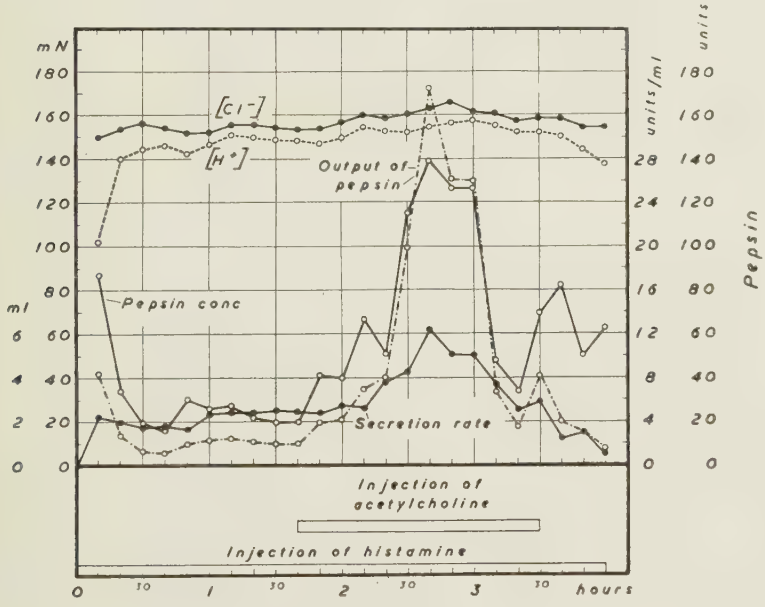

Fig. 29. The potentiating effect of acetylcholine superimposed on histamine stimulation in a Heidenhain pouch dog.

Stavraky (1949) found that the output of pepsin was blocked if atropine was given before the administration of acetylcholine but not if it was given after. In four of the experiments performed by the pre-

sent author 1 mg of atropine was injected subcutaneously during the combined administration of acetylcholine and histamine. In all cases the output of pepsin decreased to very low values.

The effect on the secretory rate of the combined administration of acetylcholine and histamine in all experiments performed by the author is demonstrated in table 6.

Table 6. **The potentiating effect of acetylcholine superimposed on continuous histamine stimulation in pouch dogs.**

Type of dog	Average dose of AC mg/hour	Secretion rate before AC	Secretion rate after AC	Volume increment
H	25	2.4	6.2	3.8
H	25	2.8	6.4	3.6
H	25	2.4	6.2	3.8
H	25	2.1	3.6	1.5
P	30	1.8	2.3	0.5
H	30	4.4	5.0	0.6
H	30	1.7	2.8	1.1
P	35	2.8	3.4	0.6
H	50	2.7	4.3	1.6
H	50	2.9	4.0	1.1
H	60	4.5	4.3	0

3. *The combined action of gastrin and acetylcholine.*

Because very little gastrin was available, only two experiments were performed, one on a HEIDENHAIN and one on a PAVLOV pouch dog.

Gastrin was first injected intravenously continuously (25 mg/hour) during 40 to 50 minutes in each dog. The secretory rate was initially 1.0 and 0.7 ml/10 minutes but decreased in both cases to 0.5 ml/10 minutes. When after that acetylcholine (40 mg/hour) was injected concomitantly during 20 to 30 minutes, the secretory rate rose to 1.6 and 1.2 ml/10 minutes. As in the experiments in which acetylcholine was administered in combination with histamine, there was in both these experiments a great increase of the output of pepsin after administration of acetylcholine.

Summary.

From the results demonstrated above the conclusion can be drawn that *when acetylcholine and histamine are administered simultaneously their combined effect is not merely additive but synergistic.* Also during combined administration of acetylcholine and gastrin there may be a synergistic effect.

Discussion.

The rôle played by the pyloric region in the cephalic phase of gastric secretion.

As mentioned above, there exists strong evidence in favour of the view that the pyloric region plays an essential rôle in the gastric phase of the secretory process. STRAATEN (1933) advanced the theory that *the co-operation of the pyloric region is essential also in the cephalic phase.* This theory was confirmed by experiments performed by UVNÄS (1942).

However, BABKIN et al. (1944) assumed that the inhibition of the gastric secretion after pylorectomy was due to hyperactivity of the sympatho-adrenal system.

Confirming the view of BABKIN et al. (1944) that such a hyperactivity is able to cause an inhibition of the vagally induced secretion, the results of the present author have shown that injection of small doses of adrenaline is able to block almost entirely the secretion produced by vagal stimulation.

BABKIN et al. (1944) found that the more crudely the stomach was handled, the greater was the inhibition. It is possible that such a crude operation as the resection of the pyloric region is able to cause hyperactivity of the sympatho-adrenal system. However, it seems farfetched to attribute the inhibitory effect after cocainization or ligation of the arteries in the experiments of the present author to such hyperactivity. Firstly, it was shown that cocainization of the main stomach had no influence on the secretory rate. Furthermore, partial ligation of the arteries of the main stomach caused no inhibition of the secretion.

The difference between the results of BABKIN et al. (1944) and those of the author may depend on the fact that dogs were used in the former investigation. However, in experiments on one dog the author found a total inhibition of the secretion after pylorectomy.

The results of the author thus indicate that *the pyloric region is probably involved in the secretory process during vagal stimulation.* However, the integrity of the pyloric region is evidently not a s i n e q u a n o n in this process. In some experiments it was possible to produce a small but distinct acid secretion by vagal stimulation even after resection of the pyloric part of the stomach.

However, this does not prove that the nervous mechanism is able to stimulate the parietal cells independently of the gastrin mechanism. It has been shown that small yields of gastrin can be obtained even from the duodenum (KOMAROW 1938; HARPER 1946).

The importance of the pyloric region to the secretion of pepsin during vagal stimulation.

Just as secretin contains secretin proper and pancreozymin (HARPER and MACKAY 1945) the pyloric mucosa may contain two substances, one stimulating the secretion of the acid solution, the other that of pepsin.

The results of the author does not disprove the existence of a humoral mechanism for the secretion of pepsin during vagal stimulation. However, the pyloric region seems to be of very little importance to this secretion. It was shown that a copious secretion of pepsin could be induced by vagal stimulation or injection of acetylcholine even after resection of the pyloric region. The stimulatory effect on the peptic cells seemed to be of about the same magnitude whether the pyloric region was resected or not.

It is therefore possible that *the peptic cells are stimulated direct by acetylcholine liberated by the vagus nerve* and that no additional stimulant is required.

Gastrin in relation to histamine.

As mentioned above, there is much evidence indicating that gastrin is not identical with histamine.

However, the results of the author, like those of KOMAROW (1942) and UVNÄS (1945), have shown that *the effect of gastrin on the gastric secretory cells is very similar to, maybe even identical with that of histamine.* Gastrin is not known to exert any physiological effect on other living tissues.

In view of these facts it is tempting to assume that the stimulatory effect of gastrin on the parietal cells is due to the liberation of histamine within these cells or close to them. This theory was first advanced by EMMELIN and KAHLSON (1944), and it was supported by experiments revealing the presence of considerable amounts of histamine in the gastric juice during injection of gastrin.

The secretory mechanism of the cephalic phase in relation to that of the gastric phase.

In this connection it may be of interest to discuss some of the principle features in the secretory mechanism of the cephalic phase in relation to that of the gastric phase.

As to *the gastric phase,* there is, as mentioned above, much evidence to prove the existence of a hormonal mechanism. Further, this hormonal mechanism is obviously able to operate in complete absence of the vagus nerve, as is made evident by the numerous studies on the effect of secretagogues and distension on vagally denervated stomach pouches.

In view of the fact that gastrin has no stimulating effect on the peptic cells, however, it seems obvious that also a nervous mechanism is normally operating in this phase of secretion. The existence of such a nervous mechanism for the control of the release of gastrin has been suggested by IVY (1941). This theory was founded on the observation that a moderate dose of atropine is able to abolish the secretory effect of secretagogues and distension acting in the stomach (LIM, IVY and MCCARTHY 1925). Atropine does not abolish the secretory response to the injection of gastrin. Therefore

it has been thought that atropine, which is known to inhibit certain kinds of nervous activity, acts by preventing the release of gastrin (Gray 1937; Ivy 1941).

As to *the cephalic phase* of gastric secretion, it seems very likely that a hormonal mechanism is active also in this phase, as indicated by the results of Uvnäs (1942) and those presented above by the author. The results of the author have also indicated that the nervous mechanism is able to operate by stimulation of the acid-producing cells in the absence of the gastrin mechanism.

However, there are facts indicating that *the nervous mechanism is a sine qua non for the acid secretion in the cephalic phase.* In a dog with a Heidenhain pouch (vagally denervated), Jemerin and co-workers (1943) showed that insulin hypoglycemia or sham feeding produced strong vagal stimulation of the main stomach, yet no secretory response occurred in the pouch.

These results may suggest that stimulation of the vagal secretory nerves does not lead to release of gastrin. However, the possibility exists that gastrin is really released but in such small amounts that the pouch does not respond. In fact it has been shown recently with a more sensitive technique that a small but distinct acid secretion can be obtained from a Heidenhain pouch in response to insulin hypoglycemia (Schofield 1950).

Summarizing, it may be said that even if the nervous and gastrin mechanisms are able to work independently of each other in the cephalic phase of secretion, it seems very probable that a co-operation is necessary to produce a strong action of the secretory glands.

The co-operation between the nervous and the humoral mechanisms in the cephalic phase of gastric secretion.

In the experiments demonstrated above it was shown that the combined action of acetylcholine and histamine on the parietal cells is more than merely additive. The same was shown to be true in the case of acetylcholine and gastrin.

These results support the view that there exists *a synergistic co-operation between the vagal secretory and the humoral mechanism of gastric secretion.* This is in accordance with the conclusion of UVNÄS (1942) that the combined action of gastrin and the vagus goes far beyond simple addition of effects.

The potentiating effect of parasympathomimetic drugs on the secretion induced by histamine is important to the understanding of the effect of vagotomy on different stimuli of the secretion. It is a well-known fact that the secretory response to histamine is reduced after vagatomy in human beings (OBERHELMAN and DRAGSTEDT 1948). Similar results on dogs have been reported (VANZANT 1947; OBERHELMAN and DRAGSTEDT 1948).

Conclusions.

Experiments on cats demonstrated above indicate that *co-operation of the pyloric part of the stomach is important to the secretory activity of the gastric vagus nerve.* It was therefore suggested that a combined neuro-humoral mechanism is necessary to produce a strong action of the parietal cells in the cephalic phase of gastric secretion, a theory advanced by UVNÄS (1942).

However, *the humoral mechanism is probably of very little if any importance to the secretory activity of the peptic cells.* These cells may possibly be stimulated direct by acetylcholine liberated by the vagus nerve.

The last statement is also in agreement with results showing that the effect of gastrin on the gastric secretory cells is very similar to, maybe even identical with that of histamine. It was therefore thought that *gastrin probably exerts its effect on the gland cells by release of histamine.*

The results obtained by combined administration of acetylcholine and histamine or gastrin indicate that *there exists a synergistic co-operation between the vagal secretory and the humoral mechanisms of gastric secretion.*

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CHAPTER VI.

General discussion.

In order to elucidate the ideas of the author, the figure below is presented (Fig. 30). The principle traits will be discussed shortly.

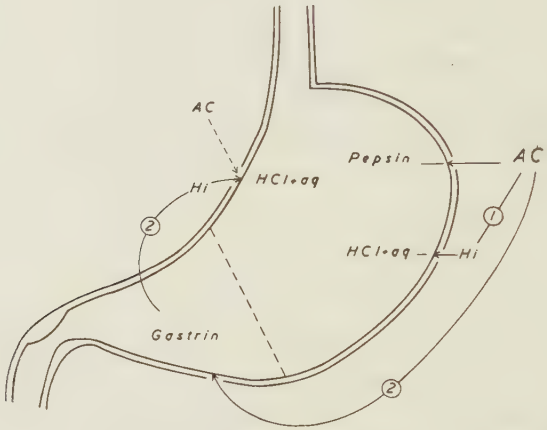

Fig. 30. The stimulation mechanisms discussed in chapter VI. In this figure AC denotes acetylcholine and Hi histamine. The dotted line to the left of the stomach indicates the synergistic action of acetylcholine on histamine

1. It has been shown that the antihistamine substance Antasten was able to inhibit the secretion induced by vagal stimulation, if Tween 20 was administered simultaneously. This fact was taken in favour of the theory that *histamine is a normal mediator in the secretory process of the cephalic phase*. This is in accordance with the hypothesis advanced by Babkin (1938) that acetylcholine, liberated at the nerve endings during vagal stimulation, causes liberation of histamine, which stimulates the parietal cells. (Cf. 1 in the figure).

To judge from the results presented in the third chapter of this work, *histamine has no stimulating effect on the peptic cells*. It is therefore probable that acetylcholine acts direct on these cells without the mediation of histamine.

2. It is rather tempting to assume that such a simple mechanism exists, but the theory cannot be accepted unreversedly. The results of the author indicate, in accordance with those of UVNÄS (1942), that *a pyloric principle,* which was supposed to be identical with EDKIN's gastrin (MUNCH-PETERSEN, RÖNNOW and UVNÄS 1944), *participates in the secretory process of the cephalic phase* (Cf. 2 in the figure). The existence of such a humoral mechanism working in the gastric phase of secretion is generally accepted.

As to the mode of action of gastrin, it was shown that the stimulating effect is very similar to, maybe even identical with that of histamine. This fact gives support to the theory advanced by EMMELIN and KAHLSON (1944) that »during the cephalic and gastric phases, parietal secretion is excited by a two-stage humoral mechanism, the first stage involving the liberation of gastrin, and histamine representing the second final link».

This theory is supported by some results of the present author. It was shown that in some cases the gastric secretion induced by vagal stimulation could be blocked almost entirely by injection of Antasten in combination with Tween 20. If, as supposed, the effect of the antihistamine drug on the gastric secretion is specific, histamine must be engaged in the vagal secretory process, whatever the mechanism may be (gastrin mechanism or not).

The activity of the peptic cells during vagal stimulation was very little influenced by resection of the pyloric region. It is possible that there exists a pepsigogue principle in the pyloric mucosa, as suggested by UVNÄS (1948). However, this principle is likely to play a very small if any rôle in the secretory process during vagal stimulation. In the opinion of the author, the peptic cells are chiefly activated directly by acetylcholine without the mediation of a humoral mechanism in the cephalic phase of secretion.

3. The theories discussed here involve two mechanisms of the parietal secretion induced by vagal stimulation, the »nervous» mechanism (acetylcholine — histamine) and the »neurohormonal» mechanism (acetylcholine — gastrin — histamine).

It is very difficult to refute the existence of any of these two mechanisms.

It is possible that the hormonal mechanism does not play the same rôle in the cephalic as in the gastric phase of secretion. This view is supported by the inability of a HEIDENHAIN pouch to secrete in response to vagal stimulation. However, in the opinion of the author the gastrin mechanism participates not only in the gastric phase but also in the cephalic phase of secretion.

The possibility of inducing a small but distinct acid secretion by vagal stimulation after resection of the pyloric region seems to indicate that the gastrin mechanism does not necessarily participate in the cephalic phase (i. e. gastric secretion can be induced according to 1 in the figure). It is possible, however, that gastrin is formed also in other parts of the gastro-intestinal canal.

Thus the results of the author does not disprove the theory of UVNÄS (1942) that the gastrin mechanism is necessary for the secretory process of the cephalic phase. According to him, gastric secretion is induced by a synergistic co-operation between gastrin and nervous impulses.

However, it must remain an open question if both mechanisms (1 and 2 in the figure) operate in the secretory process during vagal stimulation or if only the second mechanism is involved. Furthermore the possibility exists that other unknown factors participate.

CHAPTER VII.

General summary.

Introduction.

The purpose of the present investigation was to study the mechanisms by which different stimuli operate in the secretory process of normal gastric secretion. CHAPTER I (page 5).

Methods.

The experiments were carried out on HEIDENHAIN and PAVLOV pouch dogs and on narcotized dogs and cats with total stomach pouches.

Stimulating agents such as histamine, gastrin and acetylcholine were administered intravenously by continuous injection.

In other experiments secretion was induced by electrical stimulation of the vagi of the neck. CHAPTER II (page 6).

The mode of action of histamine on the gastric secretory cells.

Because of the possibility that histamine may be involved in the normal secretory process, the stimulating effect of this drug was studied.

It was shown that histamine probably acts directly on the parietal cells but has no stimulating effect on the peptic cells.

It was supposed that there is a spontaneous basal secretion of pepsin in the »resting» stomach and that this spontaneous secretion is inhibited by histamine. CHAPTER III (page 13).

Histamine as a normal stimulant of gastric secretion.

In accordance with the results of many other investigators it was found that the antihistamine substance Antasten does not per se inhibit gastric secretion.

However, in combination with the wetting agent Tween 20, Antasten was found to exert a significant inhibitory effect on gastric secretion whether induced by injection of histamine or by vagal stimulation.

These facts give evidence in support of the theory that histamine is a mediator in the secretory process of the cephalic phase. CHAPTER IV (page 41).

The co-operation between the nervous and the hormonal mechanism in the gastric secretory process.

It was shown that the pyloric part of the stomach is important to the secretory activity of the gastric vagus nerve. It was therefore suggested that a combined neuro-humoral mechanism operates in the cephalic phase of secretion.

It is true that it was possible to induce a slight *acid* secretion by vagal stimulation after pylorectomy, but a strong stimulation of the parietal cells could be produced only when the pyloric region was left intact.

On the other hand, a humoral principle localized in the pyloric region is probably of very little importance to the secretory activity of the *peptic* cells. It was assumed that these cells are stimulated direct by acetylcholine liberated at the nerve endings of the vagus nerve.

Extracts from the pyloric mucosa, which were supposed to be identical with gastrin, had an effect on the gastric secretory cells which was very similar to that of histamine. It was suggested that gastrin may exert its effect by release of histamine.

The view that there exists a synergistic co-operation between the vagal secretory and the pyloric humoral mechanism was supported by experiments showing that the combined action of acetylcholine and histamine or gastrin goes beyond a simple addition of effects. CHAPTER V (page 61).

General discussion.

The possible modes of stimulation of the gastric secretion were discussed in the light of these results. It was thought that there might be two ways of stimulating the parietal cells in the cephalic phase of secretion — by a »nervous» mechanism (acetylcholine — histamine) or by a »neuro-humoral» mechanism (acetylcholine — gastrin — histamine). None of these two possibilities could be excluded so far. CHAPTER VI (page 84).

Acknowledgements.

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